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Graduated in Food Engineering

Study of membrane emulsification process as a pre-step for the microencapsulation of lipid compounds by spray drying

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**STUDY OF MEMBRANE EMULSIFICATION
PROCESS AS A PRE-STEP FOR THE
MICROENCAPSULATION OF LIPID
COMPOUNDS BY SPRAY DRYING**

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Abstract

Food emulsions play an important role in product development and formulation, as well as to encapsulation of food additives. Conventional methods for emulsion production may present some drawbacks, such as the use of high shear stress, high energy demanding and polydisperse droplet size distribution. In this sense, membrane emulsification emerges as an alternative method to overcome all this issues and to produce fine and stable emulsions. Linseed oil has been widely studied in the last years, due to its nutritional composition, being the richest ω -3 vegetable source and for that reason it was used as the raw material for emulsion production. Premix and direct (cross flow) membrane emulsification were carried out using three different membrane materials: polysulphone, cellulose ester and α -alumina membrane. For premix membrane emulsification (PME) the variables transmembrane pressure, membrane material, surfactant type and membrane mean pore size were evaluated. The membrane mean pore size was the crucial factor to achieve emulsions by PME, once it was not possible to achieve stable emulsion with mean pore sizes lower than 0.8 μ m. For direct membrane emulsification, transmembrane pressure, surfactant concentration and cross flow velocity were evaluated by means of a experimental design. The evaluated responses were stability, droplet size and distribution and dispersed phase flux. For all the variables studied, only dispersed phase flux showed to have significant influence of pressure. Comparing both methods of membrane emulsification, premix showed to be more suitable in terms of emulsion production throughput and droplet size correlation with membrane pore size, however, in terms of stability, direct membrane emulsification showed much better results. Encapsulation of linseed oil by spray drying was promoted using the optimum point of the performed experimental design and the droplets size distribution has considerably changed with the addition of the wall material to the emulsion.

Keywords: Linseed oil, premix membrane emulsification, direct membrane emulsification, food emulsions, bioactive lipids.

Index

1	INTRODUCTION.....	1
1.1	Background and Motivation.....	1
1.2	Objectives	3
2	LITERATURE REVIEW	5
2.1	Emulsions.....	5
2.2	Membrane emulsification.....	6
2.2.1	Cross-flow ME	10
2.2.2	Premix ME	11
2.3	Parameters affecting the emulsion production	12
2.3.1	Membrane parameters.....	13
2.3.2	Process Parameters.....	14
2.3.3	Emulsion properties	15
2.4	Linseed oil	15
2.5	Encapsulation	16
3	MATERIALS AND METHODS.....	19
3.1	Materials.....	19
3.2	Emulsion preparation	20
3.2.1	Premix emulsification.....	20
3.2.2	Direct membrane emulsification	22
3.3	Membrane characterization.....	23
3.3.1	Scanning electron microscopy (SEM).....	23
3.4	Emulsion characterization.....	24
3.4.1	Viscosity.....	24
3.4.2	Density.....	24
3.4.3	Microscopy	24
3.4.4	Particle size distribution.....	24
3.4.5	Emulsion stability	25
3.4.6	Statistical analyses	25
3.5	Microencapsulation by spray drying.....	25
4	RESULTS AND DISCUSSION	27
4.1	Premix Membrane Emulsification (PME)	27

4.2	Direct Membrane Emulsification (DME).....	41
4.3	Comparison between emulsification methods	51
4.4	Microencapsulation by spray dryer	55
5	CONCLUSION AND FUTURE PERSPECTIVES	58
6	REFERENCES.....	60

Index of figures

Figure 1.	Membrane emulsification systems for controlling hydrodynamic conditions near the membrane surface (Vladisavljevic & Williams, 2005).	9
Figure 2.	The forces acting on the droplets during membrane emulsification (Hancocks <i>et al.</i> , 2013).	10
Figure 3.	Schematic diagram of cross-flow membrane emulsification (Adapted from Liu <i>et al.</i> , 2010)	11
Figure 4.	Schematic diagram of premix membrane emulsification (Adapted from Liu <i>et al.</i> , 2010).....	12
Figure 5.	Effect of wall shear stress on droplet size in oil-in-water emulsions produced using ME	15
Figure 6.	Electron micrograph of flat membranes, cellulose nitrate (a), cellulose acetate (b) and mixed cellulose ester (c).....	19
Figure 7.	Electron micrograph showing the ceramic membrane layers on top of a more open support layer (Source: PALL corporation, Membralox® Ceramic Membrane Products)	19
Figure 8.	Schematic representation of the premix membrane emulsification with agitation	21
Figure 9.	Schematic representation of the premix membrane emulsification without agitation	21
Figure 10.	Schematic representation of the direct membrane emulsification	22
Figure 11.	Mini <i>spray dryer</i> Buchi Modelo B-190.....	26
Figure 12.	Evolution of the permeate fluxes along premix membrane emulsification process with 0.1µm polysulphone (PST80)	27
Figure 13.	Hydraulic permeability before and after the PST80 process	28
Figure 14.	Retentate and permeate fraction of premix emulsification using polysulphone (0.1µm) membrane.....	28
Figure 15.	Evolution of the permeate flux during the premix emulsification processes with cellulose ester membranes (CN and CA) and Tween 20 (T20) and Tween 80 (T80) as surfactants	30
Figure 16.	Phase separation (left) and formation of a thick oil layer on the membrane surface (right) for cellulose ester membrane emulsification for the processes CNT20 (a), CNT80 (b), CAT 20 (c) and CNT 80 (d).	31
Figure 17.	Transversal (a) and frontal (b) SEM images of new (on the left) and used (on the right) cellulose acetate membrane with mean pore size of 0.45 µm.....	32
Figure 18.	Transversal (a) and frontal (b) SEM images of new (on the left) and used (on the right) cellulose nitrate membrane with mean pore size of 0.22 µm.....	33
Figure 19.	Flux of emulsion using cellulose acetate and Tween 20 as surfactant with (CAT20B) and without (CAT20) pretreatment with surfactant solution.	34
Figure 20.	Comparison between the permeate fluxes obtained with agitated and non-agitated module. ...	35
Figure 21.	Photomicrograph of coarse emulsion (left) and emulsion achieved by process AMCT20 (right).	36
Figure 22.	Stability of linseed oil emulsion prepared by PME 1.	37
Figure 23.	Flux against pressure for processes PME1, PME 2, PME 3.	38
Figure 24.	Photomicrograph with 400x magnification of emulsion prepared by PME 1 (a), PME 2 (b) and PME 3 (c).	38
Figure 25.	Droplet size distribution of processes PME 1, PME 2 and PME 3.....	39
Figure 26.	Particle size distribution of the process PME 2 accomplished on cycles.....	40
Figure 27.	Hydraulic permeability of ceramic membrane with mean pore size of 0.2µm.....	41

Figure 28. Pareto chart for the dispersed phase flux in cross flow membrane emulsification.	43
Figure 29. Droplet size distribution of linseed oil emulsions produced by cross flow membrane emulsification.	44
Figure 30. Pareto chart for surface weighted mean diameter in cross flow membrane emulsification	45
Figure 31. Pareto chart for volume weighted mean diameter in cross flow membrane emulsification	45
Figure 32. Pareto chart for span in cross flow membrane emulsification	46
Figure 33. Separation of linseed oil cross flow membrane emulsification	47
Figure 34. Pareto chart for % of phase separation in cross flow membrane emulsification	47
Figure 35. Photomicrographs of linseed oil cross flow membrane emulsification in the first day (left) and twenty-first (right)	51
Figure 36. Photomicrographs of Ultra-Turaax emulsification (a), premix ME (b) and direct ME (c)	53
Figure 37. Droplet size distribution for emulsion achieved by Ultra-Turrax, direct ME and premix ME	53
Figure 38. Stability for 7 days of emulsion achieved by turaax emulsification (a), premix ME (b) and direct ME (c)	54
Figure 39. Droplet and particle size distribution for emulsion and capsules achieved by spray dryer	55
Figure 40. Microphotographs of capsules achieved by cross flow membrane emulsification and spray dryer with magnification of 4000 X(a) and 10000 X.	56

Index of tables

Table 1 . Membrane emulsification studies	8
Table 2 . Description of emulsion formulations and process parameters	20
Table 3 . Codified independent variables	23
Table 4 . Results obtained for premix membrane emulsification performed with agitated module.....	36
Table 5 . Surface weighted (D[3,2]), volume weighted D[4,3] and span values for processes PME 1, PME 2 and PME 3.	39
Table 6 . Flux and droplet size parameters for the process PME 2 accomplished on cycles	40
Table 7 . Flux, droplet size and stability results for linseed oil cross flow membrane emulsification	42
Table 8 . ANOVA analisys of adjusted model for flux.	43
Table 9 . Emulsion flux by Premix and direct ME.....	52
Table 10 . Span, Sauter and De Brouckere mean diameter for emulsion achieved by Ultra-Turrax, direct ME and premix ME,	54
Table 11 .Span, Sauter and De Brouckere mean diameter for emulsion and capsules achieved by spray dryer.	56

1 INTRODUCTION

1.1 Background and Motivation

Nowadays, it is widely recognized that the creation of novel foods or the improvement of existing foods depends on a better understanding of the complex interrelationship between food structure and performance (McClements *et al.*, 2009).

In the last two decades or so the perception of food has changed from being just hunger satisfiers and taste bud entertainers to a source of healthy well-being. This increased consumers interest has led to the emergence of a specialized category of food products, commonly known as the functional foods, which relies on fortification of food products with micro-nutrients or functional ingredients from natural sources or relatively novel combination of food ingredients, such as, for example fibre added to soft drinks, fish oil added to bread or human gut bacterial cultures added to dairy foods (Day *et al.*, 2009).

The addition of ω -3 and ω -6 polyunsaturated fatty acids (PUFA) to functional food ingredients and their consumption in dietary supplements have experienced significant increases (O'Brien, 2009). These fatty acids have been associated with a variety of health benefits, such as reducing the risk of coronary heart diseases, hypertension, arthritis, and immune response disorders (Rubio-Rodríguez *et al.*, 2010). Linseed oil is a healthful and nutritive oil very rich in unsaturated fatty acids, being recognized as one of the greatest vegetable Omega-3 sources in nature, which represents about 57% of its total fatty acids (Zhao *et al.*, 2004).

There are basically two ways of adding (functional) ingredients to a product: In a soluble form as solution or insoluble form as dispersion (Patel & Velikov, 2011). In terms of bioactive lipids, both methods involve emulsion production and can be done by means of emulsion production by itself or microencapsulation.

Emulsions play an important role in the formulation of foods for production of oil in water (o/w) emulsions, e.g., dressings, artificial milks, cream liqueurs, as well as for preparation of some water in oil (w/o) emulsions, e.g., margarines and low fat spreads. Some emulsions are end products themselves, e.g., coffee creamers and cream liqueurs are relatively simple emulsions which remain stable towards creaming and coalescence during their production and shelf-life. Besides, emulsions can also be used as ingredients, which participate in forming the structures of more complex products. For example, yoghourts and other gelled systems contain emulsion droplets that must interact with other food ingredients, but that must not be destabilized in the process. Finally, the emulsion droplets may create new structures, i.e., in ice-cream or whipped products, where the emulsion is itself required to destabilize as a means to creating structure in the product (Charcosset, 2009). And thus, functional ingredients could be used as the raw material to obtain functional products.

The insoluble form of adding functional ingredient is the microcapsules achievement. Microencapsulation of oils in a polymeric matrix is an alternative to protect unsaturated fatty acids against lipid oxidation, thus increasing their shelf life. Besides, it also offers the possibility of controlled release of lipophilic functional food ingredients and can be useful for supplementation of foods. Moreover, it helps in masking the odor and/or taste of encapsulated materials; and simplifies the

handling, storage, and delivery of the powder-like materials produced. In the food industry, the most widely employed technologies for encapsulating lipophilic compounds are based on the production of an oil-in-water (O/W) emulsion which is then followed by either spray drying, freeze drying, molecular inclusion, enzymatic gelation, or coacervation. Of these techniques, the most common way of producing encapsulated oil is by spray drying the emulsion because it is a very efficient and flexible process that quickly removes water by vaporization and that can be carried out with readily available equipment (Ramakrishnan *et al.*, 2012).

Implementing additional functional ingredients to food products often leads to problems ranging from formulation difficulties, taste issues, product stability, product appearance and decreased bio-accessibility. What makes it even more challenging is the interaction of these functional ingredients with the complex product matrix (Patel & Velikov, 2011).

Many different emulsification methods have been developed, mostly depending upon the product (and economical) requirements. Conventionally, the emulsions are prepared by mechanical disruption of the dispersed phase droplets into the continuous phase. Colloid mills, rotor stator systems, high-pressure homogenizers, and ultrasonic homogenizers are popular types of equipments for this, due to their high throughput. Although these systems result in stable emulsions, they have high energy requirements, apply shear and extensional stresses to the product that may cause loss of functional properties of heat and shear sensitive components. In addition, they show poor control over droplet size and distribution (Joscelyne & Trägårdh, 2000; Charcosset, 2009; Nazir *et al.*, 2010).

The size and uniformity of emulsion droplets are of critical importance, since they determine the stability against coalescence and fitness in application to some extent (Zhou *et al.*, 2009), being fine emulsions more stable than the coarse emulsions. Besides, the droplet size distribution affects many of the physicochemical emulsion properties (Charcosset, 2009).

There are several advantages to produce fine emulsions: Systems with fine and well controlled particle/droplet size reduces the sensitivity to the oil with respect to the human taste sensation, and it would reduce the effect of an emulsion or microcapsule on the taste of the food itself (Shima *et al.*, 2004; Patel & Velikov, 2011). In addition, because w/o food products (e.g. margarine) contain flavouring components in the dispersed phase, the droplet size distribution affects the flavour of such products. Furthermore, it may also have a great effect on growth bacteria. When the droplet diameter is large, bacteria multiply more easily than for smaller droplet diameter, as the bacterial growth is reduced due to the lack of nutrients inside the droplets (Charcosset, 2009).

As this parameter is direct correlated to emulsion stability, it is also one of the most important parameter that affects the microencapsulation process. In general more stable emulsions results in greater encapsulation efficiency, in other words, in smaller amount of non-encapsulated material on the particle surface (Minemoto *et al.*, 2002; Barbosa *et al.*, 2005).

Some studies have shown that the method for emulsion production plays a key role in optimizing the oil encapsulation efficiency, once it affects emulsion droplet size (Ramakrishnan, 2012). Thus, over the last 20 years or so, there has been an increasing interest in using microstructured systems like a technique for making emulsions, known as 'membrane emulsification' (ME). This technique is highly attractive once it can be used to prepare emulsions with low

mechanical stress (Schröder *et al.*, 1998) or even without any shear (Kosvintsev *et al.*, 2008), being relatively simple, with considerably lower energy requirement than traditional processes, lower surfactant requirement and narrow droplet size distribution (Joscelyne & Trägårdh, 2000; Van Der Graaf *et al.*, 2005; Charcosset, 2009; Dragosavac *et al.*, 2012). Moreover, this technique allows the emulsion formation by means of two methods: crossflow or direct emulsification, which is based in a primary homogenization where the dispersed phase is pressed to pass through the membrane while the continuous phase flows on the other side of the membrane, and premix emulsification, which is based in a secondary homogenization, since it consists in the reduction of the droplet size of the already formed coarse emulsion.

Membrane emulsification has as a great advantage, the possibility to be applied to both kinds of compounds, lipophilic by means of O/W emulsions (Trentin *et al.*, 2011; Ramakrishnan *et al.*, 2012) and hydrophilic by means of water-in-oil-in-water (W/O/W) double emulsions (Shima *et al.*, 2004; Vladisavljevic *et al.*, 2004; Van der Graaf *et al.*, 2005).

A lot of work has been carried out on membrane emulsification on the last 20 years. However just in the last 15 years with the introduction of premix membrane emulsification, by Suzuki and coworkers (1998) more types of membranes has been studied for this application besides Shirasu Porous Glass (SPG). This membrane has been widely used in many research fields (Vladisavljevic *et al.*, 2003; Toorisaka *et al.*, 2003; Vladisavljevic *et al.*, 2004a; Kukizaki & Goto, 2007; Hancocks *et al.*, 2013), but it would be potentially unsuitable for food production due to its fragility, possibility of glass contamination in the product and other factors (Hancocks *et al.*, 2013). In this sense, other membrane types with narrow pore size distribution are being used, such as etched nickel film and micro-sieves with engineered pores often made using silicon (Wagdare *et al.*, 2010; Dragosavac *et al.*, 2012; Nazir *et al.*, 2013) however these membranes have high cost and they are potentially prohibitive to scale-up.

Despite of this, there are few works in the literature reporting the application of different membrane materials with small pore size for membrane emulsification. Furthermore, most of the studies in this field is devoted to encapsulation in liquid media, aiming just the entrapment of the material on the lipid matrix. Only one recent study (Ramakrishnan *et al.*, 2012) focused on the production of microcapsules by combining membrane emulsification with spray drying.

Thus, the aim of this work was to study two types of membrane emulsification processes (premix and cross-flow emulsification), as a pre-step for microencapsulation of linseed oil by spray drying.

1.2 Objectives

The objective of this work was the encapsulation of linseed oil by means of a combination of membrane emulsification processes using commercial (unstructured) membranes and drying by atomization (spray drying) in order to obtain stable emulsions and powders.

The specific objectives are:

- To evaluate the use of different membranes in the premix emulsification;

- To study the effect of surfactant type, transmembrane pressure, pore size and presence or absence of agitation on the premix emulsification;
- To understand the behavior of emulsion in each cycle of multi-stage premix membrane emulsification;
- To verify the influence of surfactant concentration, cross flow velocity and transmembrane pressure on the dispersed phase flux of direct membrane emulsification;
- To characterize the emulsions produced by both methods in terms of creaming stability, droplet size and its distribution;
- To compare direct and premix emulsification methods;
- To produce solid linseed oil microcapsules using spray drying;

2 LITERATURE REVIEW

2.1 Emulsions

Emulsions are disperse systems of two immiscible or poorly miscible liquid phases. Examples for emulsions paints, spreads, cosmetic creams, pharmaceutical ointments and sauces. Food emulsions examples are mayonnaise, which is small oil droplets dispersed in a continuous water phase (oil-in-water emulsion, o/w) or margarine consisting of small water droplets dispersed in a continuous oil phase (water-in-oil emulsion, w/o). Moreover, emulsions play an important role in the formulation of various other products as ingredients like salad dressings, artificial milks, cream liqueurs, sausage, pate, etc. Above that, it is possible to disperse a primary emulsion, e.g. a w/o emulsion in a continuous phase, e.g. water, which results in the formation of a double emulsion (water-in-oil-in-water emulsion, w/o/w). This type of emulsions contain small primary water droplets within larger oil droplets while the oil droplets are dispersed within the secondary continuous water phase. In the second step of the emulsification process, when the W/O/W droplets are produced, carefully controlled shear needs to be applied as there is a requirement not to rupture the primary emulsion (Lambrich & Schubert, 2005; Dragosavac *et al.*, 2012).

Macro emulsions are thermodynamically and intrinsically instable and tend to destabilize due to several effects. Coalescence, an irreversible process, leads to the formation of larger droplets due to the fusion of two or more droplets. Coalescence may occur in the emulsification process or thereafter and can be reduced by specific ingredients namely surfactants or surfactants, which are chemical substances (or a mixture of substances) that occupy the interface between the oil and the water phase, forming protective layers and thus stabilizing the droplets against coalescence and/or aggregation and reducing the interfacial tension. The dynamics depend on the molecular structure of the surfactant. Depending on the emulsion to be produced and the surfactant type used, the surfactant can be diluted in the oil or the water phase (Joscelyne & Trägårdh, 2000; Dickinson, 2003; Lambrich & Schubert, 2005; Schoën *et al.*, 2013).

Conventional emulsification processes such as rotor/stator high shear mixers and high-pressure homogenizers produce a bulk emulsion by comminution of the dispersed/internal phase. Despite their widespread usage these “top-to-bottom” approaches are associated with a number of disadvantages. For example, droplet size and size distribution are not well controlled, which makes batch-to-batch consistency very difficult to achieve. In addition such mechanical methods require large inputs of energy and subject the emulsion to high shear and thermal stresses which may have undesirable effects on sensitive ingredients, such as micronutrients, proteins and starches. Alternatively such high stresses may have effects on the emulsion microstructure under processing itself; e.g. processing of shear sensitive structures such as double emulsions (Spyropoulos *et al.*, 2011). In order to increase the efficiency of the emulsification process, many different methods for emulsification have been developed and adapted to special requirements over the years (Lambrich & Schubert, 2005). Membrane emulsification offers an alternative to these droplet break-up systems, as emulsion droplets in this case are formed individually/one-at-the-time. There are a number of inherent advantages to such “bottom-up” approach, namely the greatly reduced energy input required for

processing, the much lower level of shear that the system is subjected to, and also the fact that far greater control over the formed emulsion microstructure can be achieved (Spyropoulos *et al.*, 2011).

Schubert (1997) has compared the performance of different types of continuous emulsification equipment, namely high pressure homogenizers, rotor-stator systems and membrane emulsification, in terms of energy densities E_v (J m^{-3}) given the criteria of smallest drop size and narrowest distribution. In all cases higher energy densities were needed to produce smaller droplets. Droplet size ranged from about $100\mu\text{m}$ down to $0.2\mu\text{m}$. For membrane systems the droplet size increased for a given energy density as the concentration of dispersed phase (expressed as volume fraction) increased from 0.05 to 0.8. Energy densities for membrane emulsification (between 10^3 and 10^6 J m^{-3}) were some 100 times less than those demanded by high pressure homogenization (between 10^5 and 10^8 J m^{-3}) and some 10 times less than rotor-stator methods (range of 10^5 – 10^7 J m^{-3}). At a similar energy density, membranes produce smaller droplets, because less energy is lost as heat. However, if the dispersed phase viscosity and/or volume fraction is large, emulsification systems based on turbulent flow are more suitable. Droplet size distributions of turbulence-based methods tend to be broad given the nature of turbulence generation, making them more sensitive to creaming and Ostwald ripening.

2.2 Membrane emulsification

Membrane processes have become major tools in the food processing industry over the last 25 years, with the classical reverse osmosis, nanofiltration, ultrafiltration, and microfiltration processes. Membrane systems are particularly suitable for large scale production because they are easy to scale-up, by adding more membranes to a device. The main applications of membranes are the dairy industry (close to 40%, of which over 10% are used for milk protein standardization), followed by beverages (wine, beer, fruit juices, etc.) and egg products (2%). Other fields are emerging: fruit and vegetable juices and concentrates, waste streams, co-products (recovery and recycling of blood plasma in abattoirs), and technical fluids (brines, cleaning-in-place solutions). The membrane emulsification process is also expected to gain an increasing interest in the food processing industry (Charcosset, 2009).

The concept of membrane emulsification has been around for many years, but the capabilities of the process are yet to be fully utilized and explored, particularly for use in the food industry where membrane emulsification is currently considered too low throughput for large-scale production (Gijssberten-Abrahamse *et al.*, 2004). As far as the authors concern, just Morinaga Company in 1996 experimented with membrane emulsification to launch a low fat spread based on this technology (Schroën *et al.*, 2012). However, membrane emulsification may be more suitable for use in producing of individual ingredients with carefully controlled structural properties (controlled droplet size, double emulsions for applications such as in healthier foods like reduced salt or fat content products or the pharmaceutical industry (Hancocks *et al.*, 2013).

The first investigation on using membrane emulsification can be traced back to the later 1980s when Nakashima and Shimizu fabricated a particular glass membrane, called Shirasu Porous Glass (SPG), and successfully produced highly uniform-sized kerosene-in-water and water-in-kerosene

emulsions by means of cross-flow membrane emulsification. Since this time the method has continued to attract attention due to its effectiveness in producing narrow droplet size distributions at low energy consumption. More recently (1998), premix membrane emulsification was introduced by Suzuki and co-workers as an alternative technique of membrane emulsification based on direct membrane emulsification.

Membrane emulsification involves using a low pressure to force the dispersed phase to permeate through a membrane, forming fine emulsions with a uniform pore-size distribution. The distinguishing feature is that the resulting droplet size is controlled primarily by the choice of membrane and not by the generation of turbulent droplet break-up. The technique is highly attractive given its simplicity, potentially lower energy demands, need for less surfactant and the resulting narrow droplet-size distribution (Joscelyne & Trägårdh, 2000).

The mechanism of droplets formation in membrane emulsification is quite different and involves two stages: droplet growth (when the droplet inflates at the pore tip) and droplet detachment (when the droplet breaks off and then moves away from the pore tip) (Charcosset, 2009).

Although the majority of investigations on using membrane emulsification have been undertaken by Japanese workers, other novel research is being carried out in European countries, but until now, no work on this field has been carried out in Latin America. To date, in addition to experimentation using SPG membranes, the most commonly used membranes for oil-in-water emulsions, investigations of a broad range of other types of membranes, such as ceramic, metallic, polymeric and microengineered devices, have been reported (Vladisavljevic & Williams, 2005). Some of the tests performed during these years are summarized in Table 1.

Table 1 . Membrane emulsification studies

Membrane material	Mean pore size μm	Flux ($\text{m}^3 \text{m}^{-2} \text{h}^{-1}$)	Pressure (kPa)	System	Authors
Flat PTFE	1.0	up to 9	-	Premix dead end	Suzuki <i>et al</i> , 1998
Flat PTFE	1.0	1 - 5.5	100-800	Premix dead end with phase inversion	Suzuki <i>et al</i> , 1999
Tubular α -alumina	0.2; 0.5	0,007-0.15	20 or 40	Direct cross-flow	Joscelyne & Trägårdh, 1999
Tubular zirconia coated	0.1	0.01 or 0.14	100 or 250	Direct cross-flow	
Flat PTFE	1.0	2-18	-	Premix dead end, multistage (n=1–3)	Altenbach-Rehm <i>et al</i> , 2002
Tubular SPG	1.1	1,6	-	Premix dead end, multi-stage (n=3)	Toorisaka <i>et al</i> , 2003
Flat cellulose Cellulose acetate	0.2; 0.45; 0.8; 3	Not specified	300-440	Premix dead end	Shima <i>et al</i> , 2004
Tubular SPG	0.4-6.6	0.0012-0,08	5-50	Direct cross-flow	Vladislavljevic <i>et al</i> , 2004a
Tubular α -alumina	1.4 and 0.5	0.009-0.024	5-140	Direct cross-flow	
Tubular SPG	10.7	0.85 – 37	20-150	Premix dead end, multi-stage (n=1-5)	Vladislavljevic <i>et al</i> , 2004b
Tubular α -alumina	1.5	0.42-0.62	200	Premix stirring	Jing <i>et al</i> , 2005
Flat polycarbonate	-	3.7-14.7	-	Premix dead end, multi-stage	Yafei <i>et al</i> , 2006
Tubular stainless Steel	100	-	0.3	Direct rotating	Vladislavljevic & Williams, 2006
Tubular assymmetric SPG	0,67	0.011 - 0.039	35-120	Direct cross-flow	Kukizaki & Goto, 2007
Tubular SPG	8.0	70 at n=5	100	Premix dead end, multi-stage	Surh <i>et al</i> , 2007
SPG	5.0	-	120	Premix continuous	Li & Sakaki, 2008
Tubular SPG	8.0	1 - 30	100-150	Premix dead end multistage(n=1-5)	Surh <i>et al</i> , 2008
Tubular SPG	4,8	0,05	90	Direct cross-flow	Gutiérrez <i>et al</i> , 2009
Tubular SPG hydrophilic	0.2; 0.4	0.03; 0.04	600	Direct cross-flow	D'oria <i>et al</i> , 2009
Tubular SPG hydrophobic	0.4; 1	0.06; 0.84	600	Direct cross-flow	
Tubular SPG	5.4; 7.6; 9.9 and 14.8	11.8 - 114.2	25 - 200	Premix dead end	Kukizaki, 2009b
Flat Nylon	0.8	10.8 – 36	300-900	Premix dead end multistage (n=4-5)	Trentin <i>et al</i> , 2010
Flat PES	0.8	3.6 – 129	300-900		
Flat Nitrocellulose mixed ester	0.8	7.2-45	300-900		
Flat Nickel	10; 20; 30 or 40	0.3 – 3.2	-	Premix and crossflow with stirring	Dragosavac <i>et al</i> , 2012
Flat Polycarbonate	3; 5	2.304 – 16.2	manual	Premix dead end, multi-stage (n=1-3)	Cheetangdee & Fukada, 2012
Flat nickel sieves	7.1-13.2	-	50-200	Premix dead end multistage(n=1-5)	Nazir <i>et al</i> , 2013
Tubular SPG	0.2-10	-	60	Direct cross flow	Hancocks <i>et al</i> , 2013
Tubular titanium oxide	0.5-10	-	60	Direct cross flow	
Tubular polymeric	1 and 1.5	-	60	Direct cross flow	
Tubular stainless Steels steel	15	-	60	Direct cross flow	

* unit conversion was done assuming a density of the emulsions approximately 1 g/mL

Emulsions using membranes can be achieved by means of a regular droplet detachment from the pore outlets where a shear stress is generated at the membrane/ continuous phase interface by recirculating the continuous phase using a low shear pump (Fig. 1a), or by agitation in a stirring vessel (Fig. 1b). Another approach uses systems equipped with a moving membrane, in which the droplet detachment from the pore outlets is stimulated by rotation or vibration of the membrane within a stationary continuous phase (Fig. 1c). Even in the absence of any tangential shear, droplets can be spontaneously detached from the pore outlets at small disperse phase fluxes (Fig. 1d) (Vladisavljevic & Williams, 2005).

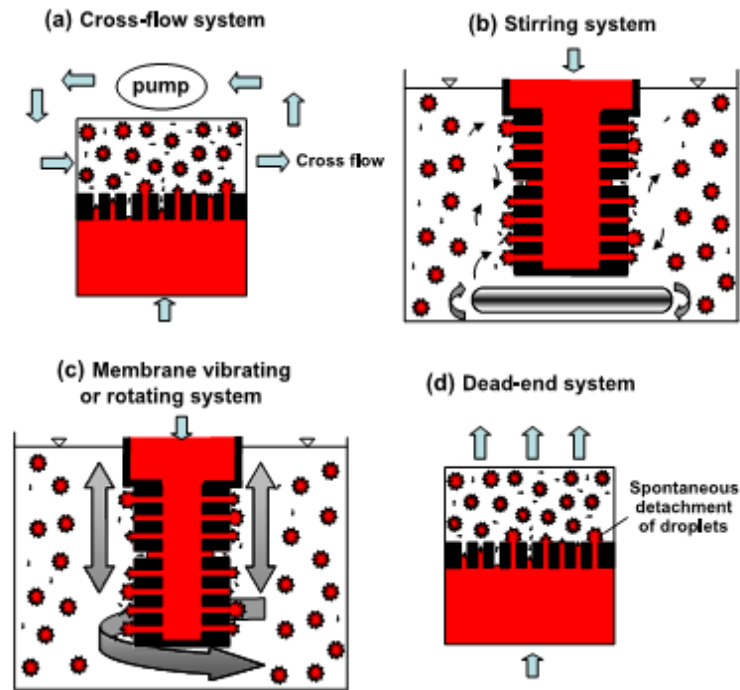


Figure 1. Membrane emulsification systems for controlling hydrodynamic conditions near the membrane surface (Vladisavljevic & Williams, 2005).

Droplet detachment at the membrane surface is known to be dependent on four main forces; shear (induced by continuous phase movement in the case of cross flow membrane emulsification, magnetic agitation or membrane movement), interfacial tension between the two emulsified fluids, inertia/pressure forces from the flow through the membrane, and buoyancy. The buoyancy force on each droplet is expected to be much smaller in magnitude than the other forces acting on droplets during detachment and can therefore be assumed to be negligible. The interfacial tension force is reduced dynamically as surfactant adsorbs at the interface between the two liquids, and is governed by the rate of this adsorption. The total reduction in interfacial tension is a function of the specific surfactant type and concentration. The applied pressure to the dispersed phase provides the inertial force of the liquid flowing through the membrane pore. An schematic representation of the forces are shown in Figure 2 (Hancocks *et al.*, 2013).

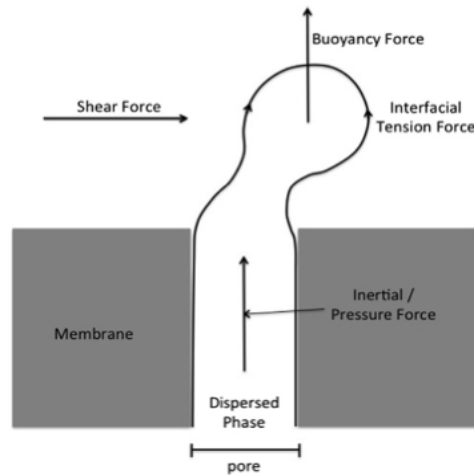


Figure 2. The forces acting on the droplets during membrane emulsification (Hancocks *et al.*, 2013).

As a rule, the dispersed phase should not wet the membrane pores, otherwise the dispersed phase will stick to the membrane and form large droplets. This means that hydrophilic membranes are suited to making o/w emulsions and hydrophobic membranes for w/o emulsions (Joscelyne & Trägårdh, 2000; Schroën *et al.*, 2012).

Membrane emulsification (ME) methods are mainly direct ME and premix. According to Schroën *et al.* (2012) in spite of many parameter studies that had been carried out on both methods, it is not possible to bring the various results for either technique together in a comprehensive framework or model, due to the numerous parameters that play a role and the complexity of the process.

2.2.1 Cross-flow ME

In cross-flow (or direct) emulsification, one phase is dispersed through a membrane into the cross-flowing continuous phase by being pressed through the pores of a membrane while continuous phase flows along the membrane surface. Droplets grow at pore outlets until, on reaching a certain size, they detach. A schematic representation of the process is shown in Figure 3. This is determined by the balance between the drag force on the droplet from the flowing continuous phase, the buoyancy of the droplet, the interfacial tension forces and the driving pressure. The droplet at a pore tends to form a spherical shape under the action of interfacial tension, but some distortion may occur depending on the flow rate of continuous phase and the contact angle between the droplet and membrane surface. The detachment of the droplets on the membrane surface results in the production of an emulsion with a narrow droplet size distribution at mild process conditions (Joscelyne & Trägårdh, 2000; Lambrich & Schubert, 2005; Nazir *et al.*, 2010).

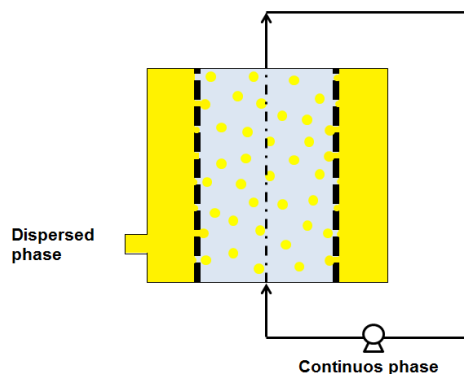


Figure 3. Schematic diagram of cross-flow membrane emulsification (Adapted from Liu *et al.*, 2010)

Cross-flow emulsification has advantages such as low and constant shear stresses along the membrane surface, low energy requirement, uniform droplet size, which allow use of less surfactant, and ease of design and scale up. A limitation in case of cross-flow emulsification is the low dispersed phase flux through the membranes (typically $0.01\text{--}0.1\text{ m}^3\text{ m}^{-2}\text{ h}^{-1}$), leading to low productivity and therefore recirculation is often required to increase the amount of dispersed phase. In that case, this recirculation may induce breakage of the droplets inside the pipes and pump, leading to a considerable polydispersity (Egidi *et al.*, 2008; Vladisavljevic *et al.*, 2012; Dragosavac *et al.*, 2012). Besides, the required membrane area is rather large, and this makes this technology expensive for large-scale application. Furthermore, it is difficult to prepare uniform emulsion droplets when the dispersed phase has high viscosity; and uniform emulsion can only be prepared using a porous membrane with very uniform pores. For 'diluted' (up to 30 vol %) specialty products that need to meet high quality standards, cross-flow emulsification is however an interesting technique to consider (McClements, 2005; Liu *et al.*, 2010; Nazir *et al.*, 2010; Dragosavac *et al.*, 2012)

Most of the published investigations for direct ME have been made using tubular micro-porous glass (MPG) membranes (Asahi Glass Company, Japan) and Shirasu porous glass (SPG). These membranes are reputed as having cylindrical, interconnected, uniform micropores. However, also Ceramic $\alpha\text{-Al}_2\text{O}_3$ (e.g., Membraflow, Germany and Membralox, SCT France) or $\alpha\text{-Al}_2\text{O}_3$ coated with titanium oxide or zirconia oxide have been used (Joscellyne & Trägårdh, 2000).

2.2.2 Premix ME

Premix membrane emulsification consists of a preliminarily emulsified coarse emulsion passing through a porous membrane. The coarse emulsion can be achieved by mixing the two immiscible phases (oil and aqueous phases) together using a conventional stirrer mixer. In most cases, a membrane is used that is wetted by the continuous phase of the premix and the emulsion is broken up into smaller droplets (McClements, 2005; Liu *et al.*, 2010; Nazir *et al.*, 2010).

If the membrane is wetted by the dispersed phase of coarse emulsion, for example, hydrophobic membrane wetted by oil phase, and suitable surfactants are dissolved in both phases, may result in a phase inversion, that is, a coarse O/W emulsion may be inverted into a fine W/O emulsion, as shown in Figure 4 (Liu *et al.*, 2010).

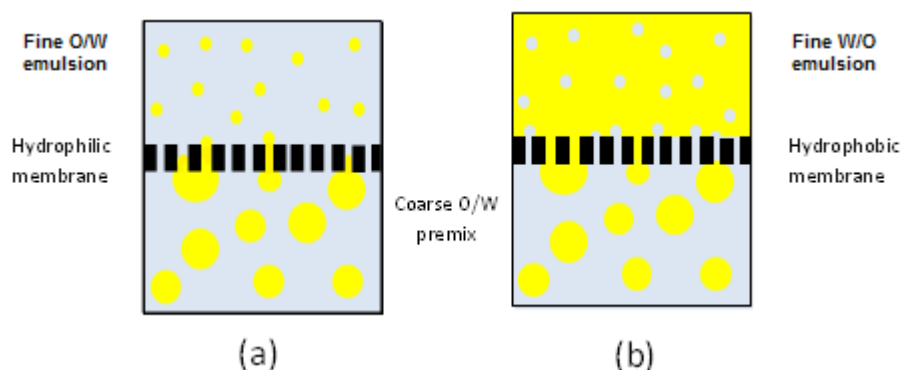


Figure 4. Schematic diagram of premix membrane emulsification (Adapted from Liu *et al.*, 2010)

Studies indicated that premix ME provides several advantages over cross-flow ME. The energy costs for premix emulsification are relatively low, since no cross-flow is needed. The energy needed can be one order of magnitude lower than for cross-flow emulsification for highly concentrated products, the optimal flux with regard to droplet uniformity is much higher than $1.0 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$, the average droplet size is smaller with the same membrane and phase compositions, the experimental set-up is generally simpler than that in direct ME; and the process parameters are easier to control. Besides, the driving pressure and emulsifier properties are not critical in the premix ME operation as in the direct ME process (Liu *et al.*, 2010).

The main drawback of premix emulsification is membrane fouling that may become serious depending on the formulation components, and related to that their interaction with the membrane and their ease of removal. Moreover, there are other disadvantages such as a higher polydispersity of emulsion droplets, since the membranes used does not have as narrow pore size distribution as SPG membranes, for that reason, in general the desired emulsion cannot be produced in a single passage (Liu *et al.*, 2010; Nazir *et al.*, 2010).

Further homogenization by repetitive cycles, commonly termed as repeated or multi-stage premix emulsification, where the coarse emulsion is repeatedly forced through the same porous membrane a number of times to achieve fine and uniform-sized emulsion droplets, yields better control of droplet size and distribution, however, at a corresponding increase of the overall energy input. In (repeated) premix emulsification, the transmembrane pressure is utilized to overcome flow resistances inside the pores and for droplet disruption to overcome interfacial tension forces (Nazir *et al.*, 2010).

2.3 Parameters affecting the emulsion production

The result the emulsification process can be judged by the droplet size distribution and the flux. For the achievement of the desired emulsion, various process controlling parameters play an important role in the efficiency of the process, affecting the droplet size formed, such as the membrane properties (pore size, pore size distribution, porosity, membrane surface type, etc.), transmembrane pressure, temperature, surfactant type and concentration, disperse phase fraction and stabilization, continuous phase viscosity, velocity of the continuous phase for direct ME and number of homogenizing cycles for premix (Joscellyne & Trägårdh, 2000; Nazir *et al.*, 2010).

2.3.1 Membrane parameters

Several authors have shown that the average droplet diameter, d_d , increases with the average membrane pore diameter, d_p , by a linear relationship, for given operating conditions:

$$d_d = c d_p \quad (1)$$

where c is a constant. For SPG membranes, values of c range typically from 2 to 10. This range was explained by differences in operating conditions, and by the type of SPG membrane used. For membranes other than SPG, the values reported for c are higher, typically between 3 and 50 (Charcosset, 2009).

Besides the pore size, an important parameter for direct membrane emulsification is the pore size distribution, once monodispersed emulsions can be produced if the membrane pore size distribution is sufficiently narrow (Charcosset, 2009). However, Zhou *et al.*, 2009 evaluated the effects of membrane parameters on the emulsification results in premix membrane emulsification and concluded that, contrary to the cross-flow membrane emulsification, the pore size distribution and the shape of pore opening did not affected the emulsification results within a wide range, once the cycles allows the production of monodisperse emulsion, being the contact angle between the membrane and surface more determinant parameter.

The contact angle was also mentioned by Gijsbertsen-Abrahamse and coworkers (2004) as the most important membrane characteristic affecting the droplet size (distribution), beside the average pore size. The wall contact angle should be as low as possible, once the membrane should be wetted by the continuous phase to obtain droplets of the disperse phase; hence the wall contact angle (measured in the continuous phase) should be smaller than 90° , characterizing by the hydrophobicity of the membrane.

Another parameter that affects the stability of the emulsion is the porosity. The closer the pores are together (at high porosities) the greater the likelihood of droplet coalescence at the membrane surface before droplets detach. Furthermore, the droplets will be deformed in the direction of flow depending on the cross-flow velocity, thus a larger distance is needed between the pores in cross-flow process than in dead end processes (Gijsbertsen-Abrahamse *et al.*, 2004). Schröder *et al.* (1998) found that a ratio of the droplet size to pore diameter of >1.6 , for a membrane porosity of 0.3, led to a significant degree of coalescence. On the other hand, if the porosity is too low then the dispersed phase flux may be insufficient for viable emulsion production (Joscelyne & Trägårdh, 2000).

The pore shape and opening also play a role in this process. Kobayashi *et al.* (2002) used two silicon membranes with different pore shape, one with oblong pores and another with circular pores. For the oblong pore he found a ratio of droplet size over (equivalent) pore size (constant c) of 2 and the ratio of droplet size over pore size was independent from the cross-flow velocity and the transmembrane pressure. With circular pores, droplet formation depended on these variables and the droplets obtained were much larger (c equal to 10).

2.3.2 Process Parameters

The transmembrane pressure (ΔP_{tm}) is the most important process parameter. It is defined as the difference between the pressure of the dispersed phase, P_d , and the mean pressure of the continuous phase:

$$\Delta P_{tm} = P_d - \frac{(P_{c,in} + P_{c,out})}{2} \quad (2)$$

where $P_{c,in}$ and $P_{c,out}$ are the pressure of the flowing continuous phase at the inlet and at the outlet of the membrane device, respectively (Charcosset, 2009). Increasing transmembrane pressure increases the flux of dispersed phase through a membrane in accordance with Darcy's law (Nazir *et al.*, 2010):

$$J = L_p \Delta P = \frac{\Delta P}{R_m \eta_w} \quad (3)$$

Where L_p is the membrane permeability, ΔP is the transmembrane pressure, R_m is the membrane resistance and η is the solution viscosity. Any deviations from Darcy's law that occur at low applied pressures are because not all pores may open (Nazir *et al.*, 2010).

It is difficult to predict emulsification pressures. In direct membrane emulsification too high pressures leads to higher throughput, but also to the risk of coalescence increases because the probability for neighbouring pores forming droplets at the same time rises. A further increase of pressure can change the regime of droplet formation, the pores generate a liquid jet instead of single droplets (Lambrich & Schubert, 2005). Too low pressures make the emulsification time long (Joscelyne & Trägårdh, 2000). When optimizing the membrane and the applied transmembrane pressure for high disperse phase fluxes, it should be kept in mind that the membrane structure affects the droplet formation time, which may affect the droplet size (Gijssbertsen-Abrahamse *et al.*, 2004).

Laouini *et al.* (2012) and Vladislavljevic' & Schubert (2003), studied the influence of the pressure applied on the dispersed phase of the SPG membrane on the flow rate through the membrane pores and the detachment of the droplets. They observed that when the dispersed phase transmembrane pressure increased, the droplet size increased while the uniformity of the emulsion decreased, thus suggesting that a high dispersed phase flow led to a poly-disperse emulsion.

Forces mainly caused by the flow of the continuous phase act on the droplet as detaching forces. Here, the flow resistance force and the dynamic buoyant force dominate the detachment process.

Another parameter with a strong influence on droplet size is the wall shear stress. Droplets formed at the membrane/continuous phase interface detach under the shear stress of the continuous phase. The characteristic parameter of the flowing continuous phase is the cross-flow velocity and its influence is often expressed in terms of wall shear stress (Pa). Typically, the crossflow velocity lies between 0.8 and 8 m s⁻¹. The droplet size decreases sharply as the crossflow velocity increases from rest and reaches a size where it becomes more or less independent of the flow velocity, shown schematically in Figure 5 (Joscelyne & Trägårdh, 1999; Joscelyne & Trägårdh, 2000; Kobayashi *et al.*, 2002; Lambrich & Schubert, 2005; Laouini, 2012). The effect of the wall shear stress on reducing droplet size is dependent on the membrane pores size, being more effective for smaller membrane pores size (Schröder & Schubert, 1998).

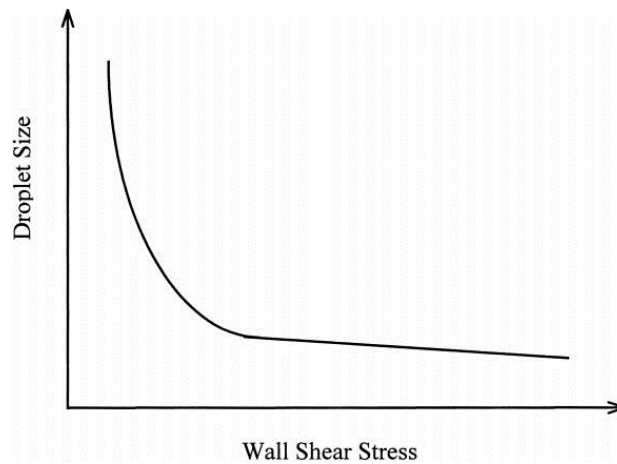


Figure 5. Effect of wall shear stress on droplet size in oil-in-water emulsions produced using ME

Temperature can be an important parameter in emulsification affecting both the viscosity of the dispersed and continuous phases and also the nature of the emulsifier as a consequence of phase inversion temperature and its solubility. However, the temperature is a parameter usually dictated by the requirements of a product (Joscelyne & Trägårdh, 2000).

2.3.3 Emulsion properties

The viscosity of the dispersed phase has an important effect on the membrane emulsification process performance. According to Darcy's law, the flux is inversely proportional to the viscosity, taking into account that for premix emulsification the viscosity is of the emulsion and in direct is the dispersed phase viscosity. If the viscosity is higher, then the flux will be lower, and as a consequence the droplet diameter will be large compared to the mean pore diameter (Charcosset, 2009).

Surfactants have two main roles to play in the formation of an emulsion. Firstly, they lower the interfacial tension between oil and water. This facilitates droplet disruption and in the case of membranes lowers the minimum emulsification pressure (Joscelyne & Trägårdh, 2000). Schröder and Schubert (1997) have suggested that the interfacial tension force is one of the essential forces holding a droplet at a pore. They found that larger droplets are produced the higher the equilibrium interfacial tension. Thus, smaller droplets are generally produced at higher surfactant concentrations. Secondly, surfactants stabilize the droplets against coalescence and/or aggregation (Dragosavac *et al.*, 2012). Besides the effect of the dynamic interfacial tension on droplet formation and on product stability, the influence of surfactants on interfaces can be reflected also in wettability changes of the surface that may occur between the interactions of the membrane and the surfactant (Schroën *et al.*, 2012).

2.4 Linseed oil

Linseed (also known as flaxseed) is an important oil crop cultivated worldwide for oil and fiber (Kasote *et al.*, 2013). It has been cultivated in more than 50 countries. Canada is the major linseed producer, followed by China, United States and India (Rubilar *et al.*, 2010).

Linseed contains about 36–40% of oil, and in recent time, linseed oil has becoming more popular as functional food in the health food market because of their reported health benefits and

disease preventive properties on cardiovascular diseases, some kinds of cancer, neurological and hormonal disorders (Oomah & Mazza, 2000; Zhao *et al.*, 2004; Herchi *et al.*, 2010). These benefits are associated with its composition. Linseed oil is a healthful and nutritive polyunsaturated oil and it is recognized as one of the richest source of α -linolenic acid (ALA), the essential fatty acid omega (ω)-3, which represents about 57% of its total fatty acids (Bozan & Temelli, 2008; Vaisey-Genser & Morris, 2003).

The low ω -3 intake in occidental diets has led to the development of nutraceuticals and functional foods in recent years, particularly those containing polyunsaturated fatty acids (PUFA) (Gallardo *et al.*, 2013). However, the high content of omega-3 fatty acid makes linseed oil highly susceptible to oxidative deterioration due to its high sensibility to heat, oxygen and light (Choo *et al.*, 2007). Thus, during processing, distribution and handling, these oils can easily oxidize, leading to the formation of unpleasant tastes and odors and, consequently, to the reduction of product's shelf life, besides promoting the generation of free radical, which may have negative physiological effects on the organism (Augustin *et al.*, 2006; Ahn *et al.*, 2008).

Microencapsulation has appeared as a key technology in delaying or inhibiting oxidation and masking undesirable odors and flavors in the final product. The process converts the oil into a free flowing powder which can be easily handled and used for nutraceuticals and/or food fortification.

Due to this increasing attention to the functional properties of this oil, microencapsulation of linseed oil has been reported in the last years by some authors (Omar *et al.*, 2009; Tonon *et al.*, 2011; Quispe-Condori *et al.*, 2011; Carneiro *et al.*, 2013 and Gallardo *et al.*, 2013) using several wall materials and some of them reaching a high encapsulation efficiency. Rubilar *et al.*, (2012) went further and applied the microcapsules achieved on the development of a soup powder enriched with microencapsulated linseed oil as a source of omega-3 fatty acids. However, none of the emulsions for the preparation of the capsules involved membrane emulsification.

2.5 Encapsulation

Encapsulation may be defined as a process to entrap at least one substance (active agent) within another substance (wall material). The encapsulated substance, except active agent, can be called the core, fill, active, internal or payload phase. The substance that is encapsulating is often called the coating, membrane, shell, capsule, carrier material, external phase, or matrix.). Two of the main capsule structures are (1) embedded particles (or core) in the shell of the capsule (matrix microcapsules), (2) a continuous shell surrounding the core (core-shell microcapsules). Core-shell microcapsules are preferred if the active agent should be released slowly over a very long time. (Vladisavljevic & Williams, 2005; Nedovic *et al.*, 2011).

Encapsulation was originally introduced in the area of biotechnology to make production-processes more efficient as the matrix around the cells allows for rapid and efficient separation of the producer cells and the metabolites. Such technologies developed approximately 60 years ago, are of significant interest to the pharmaceutical sector (especially for drug and vaccine delivery), but also have relevance for the food industry. In recent years, the food industry requires the addition of functional (or active) compounds in products. The active ingredient may be i.e., a food additive, a

medicament, a biocide, or an adhesive. A food additive may impart texture or bulk, or it may play a functional role in terms of nutritional value, food preparation or preservation. Functional ingredients include e.g., processing aids (leavening agents and enzymes), preservatives (acids and salts), fortifiers (vitamins and minerals), flavours (natural and synthetic), and spices. These compounds are usually highly susceptible to environmental, processing and/or gastrointestinal conditions and therefore, encapsulation has imposed an approach for effective protection of those (Charcosset, 2009; Vos *et al.*, 2010; Nedovic *et al.*, 2011).

Encapsulation aims to preserve stability of the bioactive compounds during processing and storage and to prevent undesirable interactions with food matrix, besides can also provide controlled release of encapsulated compounds; helps in masking the odor and/or taste of encapsulated materials; and simplifies the handling, storage, and delivery of the powderlike materials produced. Mainly, bioactive food compounds are characterized by rapid inactivation. These compounds would profit from an encapsulation procedure, since it slows down the degradation processes (e.g., oxidation or hydrolysis) or prevents degradation until the product is delivered at the desired sites (Nedovic *et al.*, 2011; Ramakrishnan *et al.*, 2012).

The most important criteria for selection of an encapsulation material are functionality that encapsulate should provide to the final product, potential restrictions for the coating material, concentration of encapsulates, type of release, stability requirements and cost constrains. Materials used for design of protective shell of encapsulates must be food-grade, biodegradable and able to form a barrier between the internal phase and its surroundings. The majority of materials used for encapsulation in the food sector are biomolecules (Nedovic *et al.*, 2011).

The most common wall materials are low-molecular-weight carbohydrate s such as sugars or maltodextrin, proteins like gelatin, and hydrocolloids such as gum Arabic or mesquite gum. The problem with some of these wall materials, such as polysaccharides, is that they lack emulsifying properties; therefore, there is the need of using an surfactant during the emulsification process. Generally, however, proteins, and whey protein in particular, can be used to stabilize emulsions and can act as effective encapsulation agents, thus providing an effective barrier against the oxidation of microencapsulated oil (Ramakrishnan *et al.*, 2012).

Many encapsulation procedures have been proposed but none of them can be considered as a universally applicable procedure for bioactive food components. This is caused by the fact that individual bioactive food components have their own characteristic molecular structure. They demonstrate extreme differences in molecular weight, polarity, solubility, etc. which implies that different encapsulation approaches have to be applied in order to meet the specific physicochemical and molecular requirements for a specific bioactive component (Ramakrishnan *et al.*, 2012).

In the food industry, the most widely employed technologies for encapsulating lipophilic compounds are based on the production of an oil-in-water (O/W) emulsion (Ramakrishnan *et al.*, 2012). On the other hand, water-soluble functional food ingredients (e.g., minerals, vitamins, flavors, enzymes, proteins, bioactive peptides, polysaccharides) can also be encapsulated using emulsions, but this time using double emulsions W/O/W by means of the entrapment of the ingredient within the internal water phase, which may have advantages for a number of applications. For example: (i)

functional ingredients could be trapped inside the inner water droplets and released at a controlled rate or in response to specific environmental triggers e.g., in the mouth, stomach, or small intestine; (ii) functional ingredients could be protected from chemical degradation by isolating them from other water-soluble ingredients that they might normally react with; (iii) water-soluble functional ingredients that have undesirable sensory qualities (e.g., bitter, astringent, or metallic flavors) could be trapped within the inner water phase so that their undesirable sensory attributes are not perceived in the mouth during mastication (Shima *et al.*, 2004; McClements *et al.*, 2009). The achievement of the emulsions is then followed by either spray drying, freeze drying, molecular inclusion, enzymatic gelation, or coacervation (Ramakrishnan, 2012).

One of the most commonly used industrial technologies for encapsulation is spray drying. It is being applied for both bioactive food molecules and living probiotics. It is a fast and relatively cheap procedure that, when adequately performed, is highly reproducible. The principle of spray drying is dissolving the core in a dispersion of a chosen matrix material. The dispersion is subsequently atomized in heated air. This promotes fast removal of the solvent (water). The powdered particles are then separated from the drying air at the outlet at a lower temperature. Thus, this process allows the encapsulation while maintaining relatively low temperature of the particles, maintaining the quality of the heat-sensitive compounds. The relative ease and also the low cost are the main reasons for the broad application of spray drying in industrial settings. The technology, however, has also some major disadvantages. The first is its small field of application. It is an immobilization technology rather than an encapsulation technology which implies that some bioactive components may be exposed (Ré, 1998; Vos *et al.*, 2010).

Emulsion plays a key role in optimizing the oil encapsulation efficiency because the emulsion droplet size distribution correlates with this parameter (Ramakrishnan *et al.*, 2012). Jafari *et al.* (2008) studied the effect of the emulsion size of fish oil droplets between 0.21 and 4.6 μm produced by different emulsification systems. Their results suggested that a smaller droplet size yields a higher encapsulation efficiency. The fact that the oil droplets have similar volumes has a positive effect on their packing inside the microcapsules, enhancing the amount of encapsulated oil (Ramakrishnan *et al.*, 2012).

The other process parameter that controls oil encapsulation efficiency is the oil-to-wall material ratio, which usually ranges from 0.1:1 to 1:1 (Tan *et al.* 2005). Recent studies show that when the oil-to-wall material ratio increases, so, too, does the oil encapsulation efficiency (Ramakrishnan *et al.*, 2012).

As far as the author's knowledge, Ramakrishnan *et al.* (2012) was the first and only to combine membrane emulsification with spray drying. In this case, this was done in order to produce fish oil microcapsules.

3 MATERIALS AND METHODS

3.1 Materials

Refined sunflower oil (Sinhá, Itumbiara, Brazil) (food grade from a local supermarket) was used in the preliminary tests in order to get a feel of the process before using the linseed oil. Then, linseed oil was purchased from O Sabor da Terra (Bragança Paulista, Brazil) was used as the active material in direct and premix emulsification. The surfactants used were: polysorbate 20, commercially known as Tween 20 (Synth, São Paulo, Brazil); polysorbate 80, commercially known as Tween 80 (Sigma–Aldrich Company Ltd., UK) and isolated whey protein (Alibra, Campinas, Brazil). The wall material used for the encapsulation was the modified starch CAPSUL[®] MHT -1944 (National Starch, Brazil).

For premix emulsification the polymeric membranes used were polysulphone supported by polypropylene (Alfa-Laval) with mean pore diameter of 0.1 μm and three membranes composed of cellulose ester, cellulose nitrate (Poretics), cellulose acetate (Millipore) and mixed cellulose ester (Whatman) without support material with mean pore diameter of 0.22, 0.45 and 0.8 μm and thickness of 100, 100 and 140 μm respectively. All the membranes used were flat. The electron micrographs with magnification of 300 X of the flat cellulose membranes are shown in Figure 6.

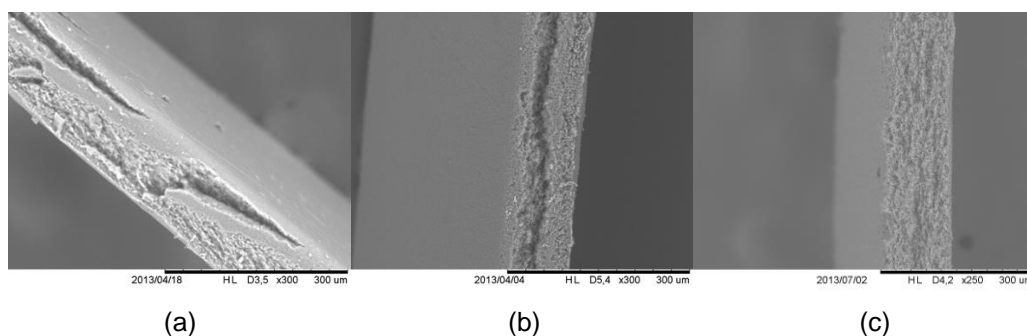


Figure 6. Electron micrograph of flat membranes, cellulose nitrate (a), cellulose acetate (b) and mixed cellulose ester (c)

For direct emulsification α -alumina membrane (Pall Corporation, New York, USA) was used with mean pore size of 0.2 μm composed by a support of the same material with porosity of 0.3 and thickness of 2mm and an active layer with porosity of 0.33 and thickness of 25 μm (Lepercq-Bost *et al.*, 2010). The ceramic membrane structure is shown in Figure 7.

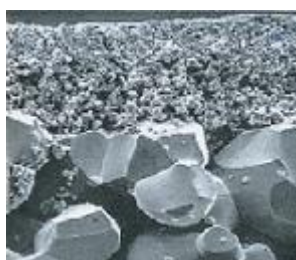


Figure 7. Electron micrograph showing the ceramic membrane layers on top of a more open support layer (Source: PALL corporation, Membralox[®] Ceramic Membrane Products)

3.2 Emulsion preparation

3.2.1 Premix emulsification

3.2.1.1 Preliminary tests

Firstly, preliminary tests were performed for premix membrane emulsification using sunflower oil. O/W emulsions were prepared in a two-step emulsification system. Firstly a coarse emulsion was prepared. The continuous phase was prepared by the addition of surfactant to distilled water at 25 °C and the mixture was stirred by magnetic agitation until completely dissolved. Then, the dispersed phase (oil) at a concentration of 20% was added to the aqueous phase by blending, using a rotor-stator blender (Ultra-turrax IKA T18 Basic, Wilmington, USA), at 15,000 rpm for 5 min.

In the preliminary tests, two different modules were used; with and without agitation in order to evaluate the influence of surface shear stress on the emulsion formation. The process conditions for each test are shown in Table 2.

Table 2 . Description of emulsion formulations and process parameters

Membrane	Mean pore size (µm)	Surfactant	Surfactant Concentration	Pressure (bar)	Agitation	Process code
Polysulphone	0.1	Tween 80	1%	6	No	PST80
Cellulose nitrate	0.22	Tween 20	2%	3.5	No	CNT20
Cellulose nitrate	0.22	Tween 80	2%	3.5	No	CNT80
Cellulose acetate	0.45	Tween 20	2%	1.0	No	CAT20
Cellulose acetate	0.45	Tween 80	2%	1.0	No	CAT80
Cellulose acetate	0.45	Tween 20	2%	1.0	Yes	ACAT20
Mixed cellulose ester	0.8	Tween 20	2%	1.0	Yes	AMCT20

The emulsion obtained previously was then loaded into the premix reservoir and pressed with nitrogen gas through the membrane. A manometer installed on the output of nitrogen cylinder measured the transmembrane pressure. The pressure applied to the system was determined by the mechanical resistance of the membrane (presence or absence of a support) and the membrane resistance to the emulsion passage (related to its mean pore size). Thus, the pressure applied was the minimum pressure observed to obtain some flux.

As the polysulphone is a supported membrane, and hence has higher mechanical resistance, and additionally it has the lower mean pore diameter, a higher pressure had to be applied in order to obtain an emulsion flux, however, no sintered plate was used, once it adds more resistance for the filtration. For the ester cellulose membranes, the sintered plate had to be used in order to promote a support and thus enhance the membrane mechanical strength. The final emulsion resulting from this process was collected in a graduated cylinder, allowing the record of volume, while the weight was measured in a semi analytical balance. In each experiment a new membrane was loaded into the module and immersed in water in order to wet the pores with the continuous phase.

The schematic representations of the modules with and without agitation are shown in Figure 8 and 9 respectively. The membrane area was different on the two modules. In the module with

agitation, the membrane diameter was 4.7 cm and in the module without agitation the membrane diameter was 8.9 cm, giving a filtration area of 0.0017 and 0.0062 m² respectively.

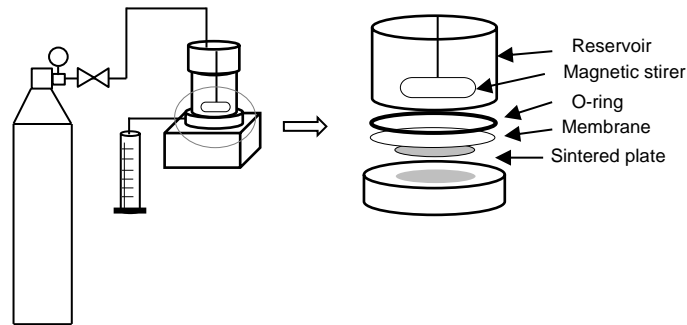


Figure 8. Schematic representation of the premix membrane emulsification with agitation

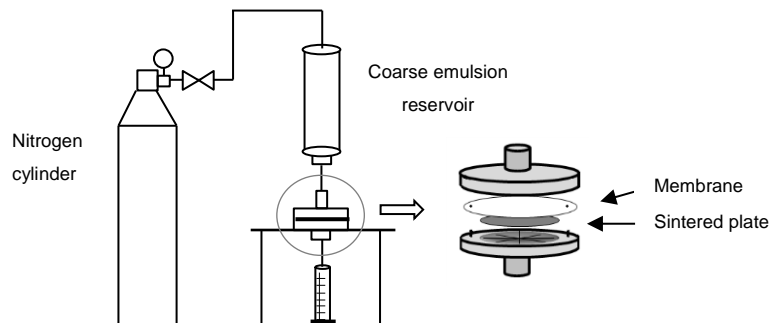


Figure 9. Schematic representation of the premix membrane emulsification without agitation

Permeate flux was calculated using volume and time data according to Eq 4.

$$J = \frac{v}{t A} \quad (4)$$

where J is the permeate flux (m³ m⁻² h⁻¹), v is the volume of permeate (m³), t is the time needed to measure this volume (h) and A is the effective membrane area (m²).

3.2.1.2 Linseed oil tests

After the preliminary tests with sunflower oil, tests with linseed oil were done only in the agitated module using the same surfactant concentration of 2%.

A test using the same pressure used for sunflower oil (1 bar) was carried out in order to understand the oil influence on emulsion production. Additionally to this test, two more tests were carried out, applying higher pressures (2 and 3 bar) to evaluate the influence of transmembrane pressure on permeate flux and emulsion stability. The membrane used for these tests was mixed cellulose esters with pore size of 0.8 μm.

In order to increase monodispersity, multi-stage premix emulsification was also carried out on the agitated module and mixed cellulose esters with pore size of 0.8 μm. This process was done in batch mode where the emulsion was hosted in the module feed stream and collected in the permeate stream. In the end of the process, the collected emulsion entered as feed in the module,

characterizing a new cycle. Cycles were performed without module disassembling and membrane cleaning.

3.2.2 Direct membrane emulsification

The continuous phase (aqueous solution) was prepared by addition of surfactant (Tween 20) in the desired concentration to distilled water at 25 °C and the mixture was stirred by magnetic agitation until completely dissolved. The dispersed phase was composed only by linseed oil and the amount was defined to have final emulsions with oil concentration of 20%.

O/W emulsions were prepared in direct emulsification cross-flow system, using integral asymmetric tubular $\alpha\text{-Al}_2\text{O}_3$ membrane. The system houses tubular membranes of 68 mm inner diameter and 250 mm in length. The system separated the inside and outside of the membrane, allowing the dispersed phase to be pressurized through the membrane from the outside of the tube using nitrogen gas, while the continuous phase flowed inside the membrane in a recirculating fashion using a piston pump, as shown in Figure 10. The pressure in the system was monitored by gauges installed on the inlet and outlet of the membrane module and on the nitrogen cylinder on the permeate side, in order to accurately control transmembrane pressure. Transmembrane pressure was defined as the pressure applied to the dispersed phase, measured at the dispersed phase tank, minus the average of the input and output pressures of the continuous phase through the membrane module. Dispersed phase flux was calculated using volume and time data according to Eq 4, but this time, v was not the permeate volume, but the dispersed phase volume (m^3) that passed through the membrane.

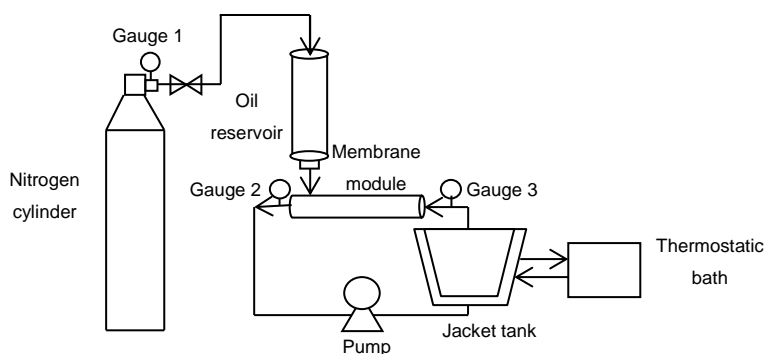


Figure 10. Schematic representation of the direct membrane emulsification

A 2^3 central composite design was used to perform the tests for the direct membrane emulsification of linseed oil, considering three factors (independent variables): continuous phase velocity (cross flow velocity), transmembrane pressure and concentration of the surfactant Tween 20. Three levels of each variable were chosen for the trials, including four replicates of the central point, giving a total of 12 combinations (Table 3). The following polynomial equation was fitted to data:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

Where β_n are constant regression coefficients; y is the response and x_1 , x_2 and x_3 are the coded independent variables (continuous phase velocity, pressure and surfactant concentration, respectively).

The responses evaluated in the experimental design were transmembrane flux; stability, in terms of % of separation; particle size, in terms of Sauter mean diameter (surface mean diameter) and De Brouckere mean diameter (volume mean diameter); and droplet size distribution (span).

The boundary conditions of the experiment design (Table 3) were determined in preliminary tests (data not shown).

The tests were performed randomly with 4 replicates in the central point, summarizing a total of 12 trials and named as CFME.

Table 3 . Codified independent variables

Code	Pressure (bar)	Velocity (m s ⁻¹)	Tween 20 concentration (%w/w)
-1	1.5	3.0	1.0
0	3.0	5.5	2.0
1	4.5	8.0	3.0

After each process, the module was cleaned by circulation with water to remove the excess of oil and disassembled to clean the parts separately. The membrane was immersed in a bath with controlled temperature of 70°C for one hour. Then, it was subjected to ultrasonication for 2 hours, since Vladisavljevic *et al.* (2004) showed that this treatment was essential to completely clean the membrane pores. Both cleaning steps were performed with a solution with concentration of 0.2 % of a commercial enzymatic detergent KOCHKLEEN® UC III (Koch Membrane Systems, USA).

The hydraulic permeability was measured before each process in order to assure that the cleaning procedure was efficient and that the membrane was in the same conditions. This parameter was calculated from Darcy's equation.

$$J_v = L_p \Delta P_{TM} \quad (6)$$

Where J_v is the water flux, ΔP_{TM} is the transmembrane pressure and L_p is the hydraulic permeability.

3.3 Membrane characterization

3.3.1 Scanning electron microscopy (SEM)

Membranes used for premix were observed before and after the process in order to evaluate the possible fouling occurrence on the membrane. This analysis was done in a Scanning Electron Detector microscope, SEM TM 3000 (Hitachi, Tokyo, Japan), operating at 15 kV. The samples were fixed directly on stubs of 12 mm diameter and then subjected to metallization (sputtering) with a double coated carbon conductive tab (PELCO Tabs™, TED PELLA, USA). After metallization, the samples were observed with high magnifications. Image acquisition was performed by the software supplied by the instrument.

3.4 Emulsion characterization

3.4.1 Viscosity

Viscosity of continuous phase and linseed oil emulsion were measured through the determination of flow curves using a rotational rheometer Haake Mars (Karlsruhe, Germany) with a range of shear rate from 0 to 500 s⁻¹. Measurements were made using stainless steel plate-plate geometry with a diameter of 35 mm and a gap of 1 mm. Temperature was controlled at 25 °C. Rheograms were analyzed according to empirical models and the emulsions viscosity was calculated as the relationship between shear stress and shear rate.

3.4.2 Density

Density measurement for emulsion produced by direct membrane emulsification was done using a pycnometer, since this apparatus is a simple and accurate mean to determine this parameter. Five replicates were done in order to reduce the analysis error.

3.4.3 Microscopy

In order to evaluate visually the droplet size and its distribution, photomicrographs of O/W coarse (for premix membrane emulsification) and fine emulsions (for both methods) were taken immediately after preparation using a Leica Microsystems (Wetzlar, Germany) optical microscope. Additionally, to evaluate the stability, photomicrographs were taken twenty first days after emulsion preparation to evaluate the apparent differences occurred along time. Droplet samples were poured into microscope slides, covered with glass cover slips and observed using the 40× and 100× objective lenses.

3.4.4 Particle size distribution

The droplet size distribution and droplet diameter was measured using a laser light diffraction instrument, Mastersizer S2000 (Malvern Instruments, Malvern, UK).

A small sample was suspended in the dispersion unit with agitation of 1750 rpm, and the droplet size distribution was obtained in triplicate by the equipment. Measurements were made in duplicate and for each sample and the volume distribution, expressed as a percentage, was based on the equivalent-sphere particle size distributions calculated from the averaged data.

There are several ways to represent particle size. As the sphere is the only shape that can be represented by a single number, there is a relation between the particle dimensions to the spheres diameters with different sizes. Thus, the particle dimensions can be expressed in terms of the diameter equivalent to the same surface area, volume or weight. The most interesting ones are mean De Brouckere diameter or volume mean diameter (D[4.3]) and mean Sauter diameter (D[3.2]) (Tonon, 2009). The first one is the weighted average volume diameter, assuming spherical particles of the same volume as the actual particles and according to Nazir *et al.* (2010) the second one is defined as the diameter of a spherical droplet having the same area per unit volume (Sv) as that of the total collection of the droplets in the emulsion:

$$D[3.2] = \frac{6}{S_v} = \left(\sum_1^{k_s} \frac{v_i}{d_i} \right)^{-1} \quad (7)$$

where v_i is the volume fraction of droplets in the i th range of sizes having mean diameter of d_i and k_s is the number of size ranges.

The size distribution data was be used to calculate coefficients of variation (spans) to indicate the width of the size distribution and consequently degree of droplet size uniformity and it was calculated using the following expression:

$$Span = \frac{D_{90} - D_{10}}{D_{50}} \quad (8)$$

where D_{90} , D_{10} , and D_{50} are the droplet diameters of 90%, 10%, and 50%, respectively, of the population on the size distribution curve.

The data acquisition was performed by the software Mastersizer 2000, version 5.31.

3.4.5 Emulsion stability

Immediately after the emulsion preparation, 50 mL aliquots of each sample were transferred to graduated cylinders of 50 mL, sealed, stored at room temperature for one month, and the volume of the upper phase measured daily. The stability was measured by % of separation and expressed as:

$$\%Separation = \left(\frac{H_1}{H_0} \right) \times 100 \quad (9)$$

Where: H_0 represents the emulsion initial height and H_1 is the upper phase height.

3.4.6 Statistical analyses

The software Statistica 8.0 (Statsoft) was used to analyze the results obtained for flux and for the emulsion characterization parameters (stability, $D[3.2]$, $D[4.3]$ and span).

3.5 Microencapsulation by spray drying

After statistic evaluation of experimental design for emulsion achievement by direct membrane emulsification, a process was chosen to be atomized by spray drying.

Direct cross flow membrane emulsification was carried out as described previously in section 3.2.2. The wall material in the proportion of 1:2 oil:wall material was added at the end of the emulsification step and mixed until dissolved, as described by Ramakrishnan *et al.* (2012).

Microencapsulation was carried out with a mini *spray dryer* Buchi Modelo B-190 (Flawil, Swiss) (Figure 11) operating at an atomization pressure of 7 bar, with drying air flow of 700 L h⁻¹. The emulsions were fed into the main chamber through a peristaltic pump and the feed flow rate was controlled by the pump rotation speed. The operation conditions in the spray dryer were air inlet and outlet temperatures of 180 and 95 °C, respectively, and an emulsion flow rate of 578 mL/h.



Figure 11. Mini *spray dryer* Buchi Modelo B-190

The particles were analyzed in terms of particle size distribution by laser diffraction and morphological analysis by Scanning Electron Microscope (SEM). The analysis description is in the topics 3.5.2 and 3.3.1 respectively.

4 RESULTS AND DISCUSSION

4.1 Premix Membrane Emulsification (PME)

The influence of membrane material and pore size on the permeate flux and stability of emulsion was studied, using the four organic membranes listed in section 3.1.

Initially, tests using sunflower oil and the module without agitation were done. The polysulphone membrane was firstly tested, since it had the smallest mean pore size ($0.1\ \mu\text{m}$). The module was used as shown in Figure 9, however without the sintered plate, since the polysulphone membrane is supported by polypropylene, and does not need an additional resistance. The applied transmembrane pressure was 6 bar and this value was verified as the minimum pressure to obtain permeate flux, based on the membrane mechanical resistance. This process showed a very low permeate flux, presenting an average value of $0.004\ \text{m}^3\ \text{m}^{-2}\ \text{h}^{-1}$ (Figure 12).

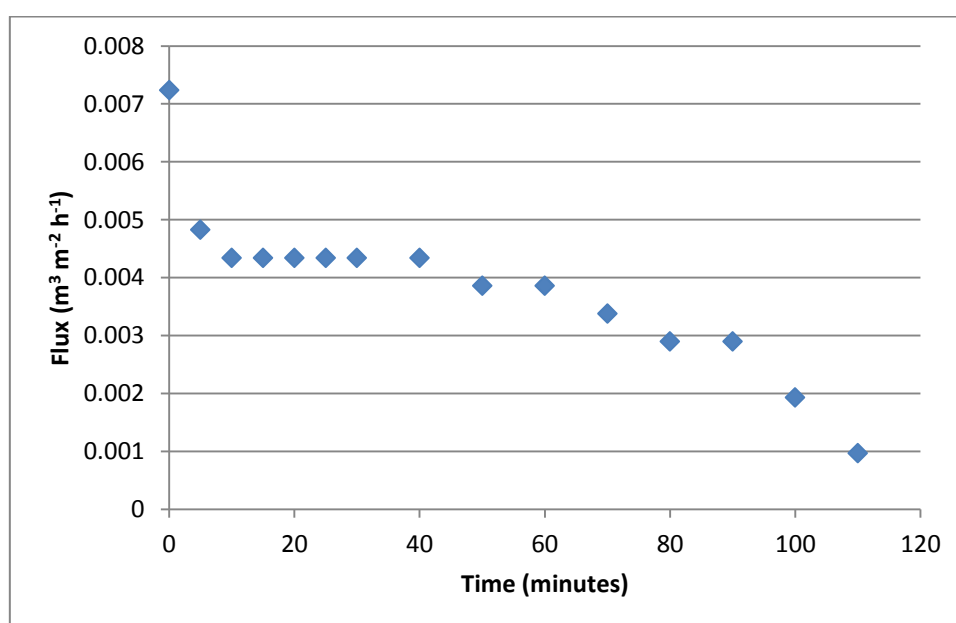


Figure 12. Evolution of the permeate fluxes along premix membrane emulsification process with $0.1\ \mu\text{m}$ polysulphone (PST80)

A flux decay was observed along the processing time. In order to quantitatively study this behavior, the water flux was measured at different transmembrane pressures before and after the emulsification tests (Figure 13) and hydraulic permeability was calculated according to Darcy's law (Equation 3). The observed decrease of the hydraulic permeability from 0.0343 to $0.0081\ \text{m}^3\ \text{m}^{-2}\ \text{h}^{-1}\ \text{bar}$ can be attributed to a cake formation on the membrane surface.

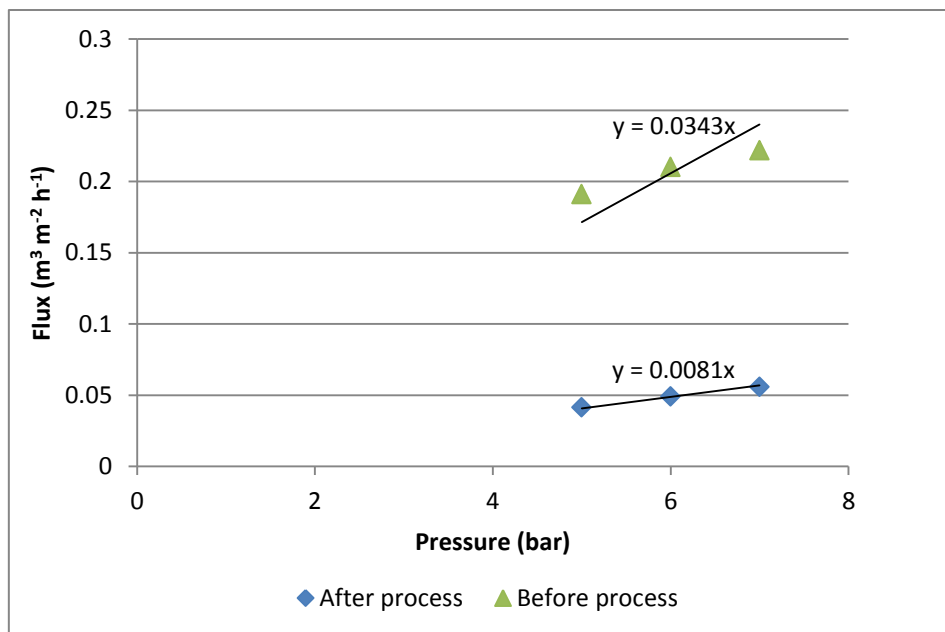


Figure 13. Hydraulic permeability before and after the PST80 process

Ideally, the process should just be finished when all the feed emulsion had permeated through the membrane, however, due to the very low flux observed in this test, it was not possible to complete the process. Thus, the retentate fraction was collected, and even just visually, it was possible to conclude that the oil has been retained by the membrane (Figure 14), and the emulsion did not permeate as expected in the premix membrane emulsification processes.



Figure 14. Retentate and permeate fraction of premix emulsification using polysulphone (0.1 µm) membrane

Koltuniewicz *et al.* (1995) affirmed that demulsification may occur if the transmembrane pressure is lower than the capillary pressure in a pore, because the oil droplets cannot pass through the pores. For this reason, three more tests were planned using higher transmembrane pressures (6, 7 and 8 bar), even the use of such high pressure has not yet been reported for membrane emulsification. However, none of them resulted in stable emulsions, always presenting oil retention and consequently, demulsification.

However, this was not completely unexpected, once demulsification is also promoted by membrane technology. According to Coutinho *et al.* (2009), polymeric membranes have been shown to be an effective means of breakage of water-in-oil emulsions,. In general, the smaller the mean pore size of the membrane the better the efficiency of components separation in the emulsion. Koltuniewicz *et al.* (1995) evaluated the microfiltration of emulsion with the same material and mean pore size membrane used in the present work for oil emulsion separation from water and reported this method as one of the most effective methods for this purpose.

According to Li *et al.* (2009) oil/water separation is very important in different technological sectors such as food, pharmaceutical, petrochemical, metallurgical, cosmetic and dyestuff industries, where large amounts of waste water containing oil are frequently generated. Thus membrane emulsification emerges as an alternative to the traditional separation techniques for oily wastewater treatment as gravity settling (API separator), skimming, dissolved air flotation, coalescence and centrifuging, since it is an inexpensive and efficient alternative to separate emulsions (Kocherginsky *et al.*, 2003; Zhou *et al.*, 2010). This method has been applied since 1994 and it is still studied to improve its efficiency, reaching oil retention of 99% according to Li *et al.* (2009).

However, for the purpose of this work, the 0,1 μm polysulphone membrane was not considered adequate to obtain fine emulsions by premix emulsification.

Ester cellulose membranes with greater pore size were tested on the same module (without agitation). Unfortunately, due to the differences on the mechanical resistances, the process conditions applied to the process were not the same as for polysulphone. Due to the fact that none of the ester cellulose membranes were supported, previous tests showed that the sintered plate was required for the process to assure the membrane integrity and to avoid its breakage. Moreover, the applied transmembrane pressure applied was also modified according to the pore size, once it was applied only the minimum pressure to obtain permeate flux, in order to avoid membrane rupture.

In order to evaluate the influence of membrane pore size (0.22 and 0.45 μm) and surfactants (Tween 20 and Tween 80) on the permeate flux, four tests were performed with similar membrane material (cellulose esters). Both surfactants are nonionic and have very similar structure, being the only difference in its hydrophobe, since Tween 20 is 20 mole ethoxylate of sorbitan monolaurate and Tween 80 is 20 mole ethoxylate of sorbitan mono oleate. Thus, Tween 20 has lauric acid and Tween 80 has oleic acid in hydrophobe.

The permeate fluxes along the process of the four tests are shown in Figure 15. The results clearly show that in all processes there was a sharp decrease on the flux in the first minutes of process. This behavior is probably due to a large influence of surface phenomena by strong physicochemical interactions between the emulsion components and the membrane. This surface phenomena results in a localized increase in concentration near the forming a cake layer, thus reducing the transmembrane flux. Moreover, Schroën *et al.* (2012), reported that strong fluxes decline may be an indication for wettability changes taking place, even being this phenomenon only observed by Vladisavljevic *et al.* (2012) for hydrophobic membranes. The average flux of CNT20, CNT80, CAT20 and CAT 80 were 0,030, 0,017, 0,019 and 0,006 $\text{m}^3 \text{m}^{-2} \text{h}^{-1}$ respectively. These fluxes are in agreement with the exposed by Joscelyne & Tragardh (2000), which observed oil fluxes for production

of o/w emulsions in the range of $0.002\text{--}0.02\text{ m}^3\text{ m}^{-2}\text{ h}^{-1}$ using hydrophilic membranes with an average pore size of $0.2\text{ }\mu\text{m}$. Cellulose nitrate presented a slightly better flux, even having smaller mean pore size probably due to the higher pressure applied in this process. Another possibility to consider is that even using the same kind of membrane material (cellulose esters), different suppliers probably deals to different fabrication methods that could result in modification on membrane structure and pores interconnectivity. However, Tween 20 showed to be more suitable for emulsion production, since it promoted better throughput. For that reason, in the following tests, only Tween 20 was used as surfactant.

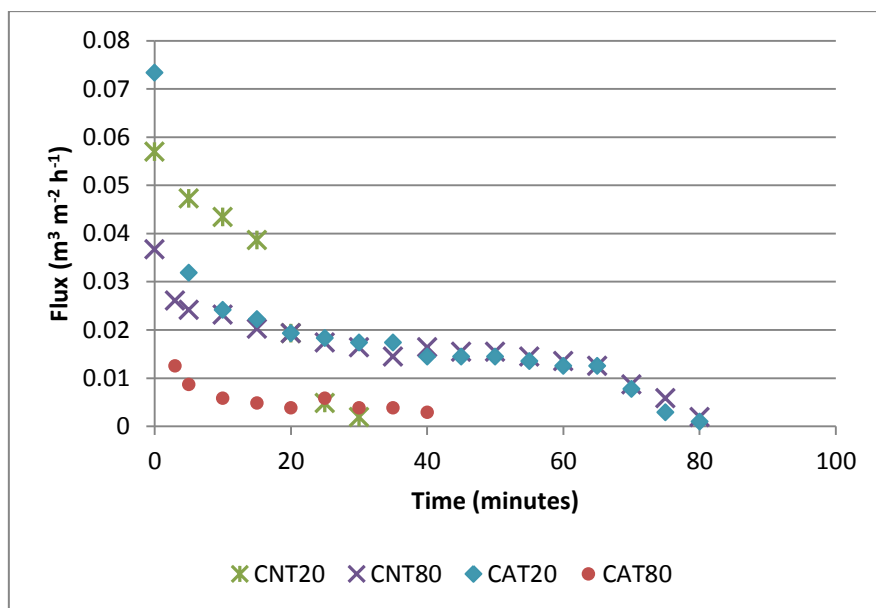


Figure 15. Evolution of the permeate flux during the premix emulsification processes with cellulose ester membranes (CN and CA) and Tween 20 (T20) and Tween 80 (T80) as surfactants

In addition to the flux, the influence of the membrane material and the type of surfactant on the emulsion stability was also studied.

For the ester cellulose membranes with pore size of 0.2 and $0.45\text{ }\mu\text{m}$, both surfactants were unable to promote stable emulsions, occurring the visually perceived phase separation just after the passage through the membrane (Figure 16). Besides, in all the experiments, a thick oil layer was formed on the membrane surface, being visibly detected. Both phenomena probably have a relationship between each other, since they indicate there is some interaction between the emulsion and the membrane that promotes this another kind of demulsification.

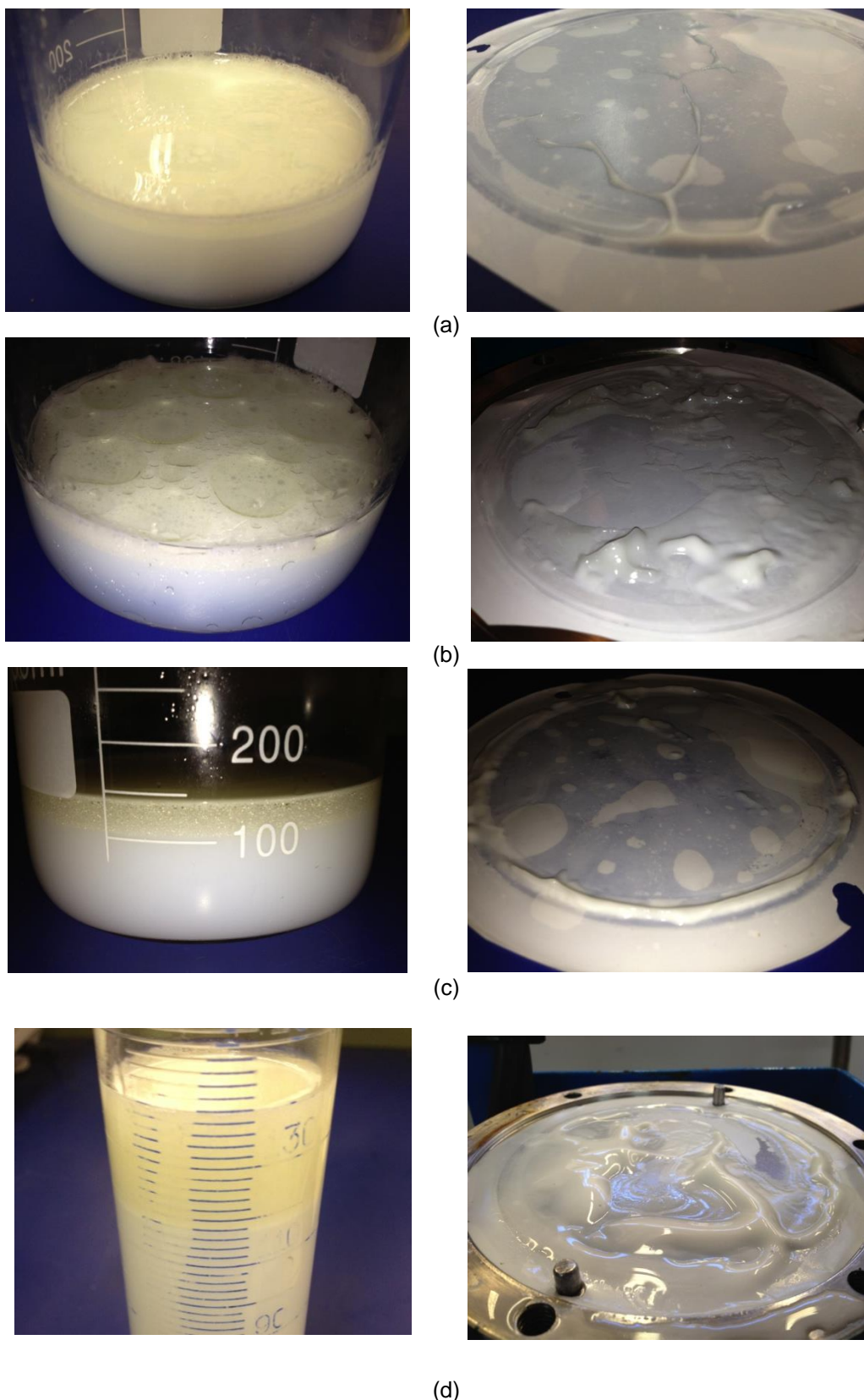


Figure 16. Phase separation (left) and formation of a thick oil layer on the membrane surface (right) for cellulose ester membrane emulsification for the processes CNT20 (a), CNT80 (b), CAT 20 (c) and CNT 80 (d).

According to Zhou *et al.*, (2010) this is a common behavior, since oil droplets stick easily on the membrane surface resulting in the formation of the cake layer. More seriously, the oil droplets can

be squeezed into and through the membrane channels even if the size of oil droplets is far larger than the pores diameter of the membrane under the applied pressure.

In order to evaluate the oil deposition on the membrane surface due to the cake layer formation and phase separation previously described, a morphology analysis in the same magnification of the membrane surface before and after the emulsification process was carried out on both ester cellulose membranes using a Scanning Electron Microscope (Figures 17 18).

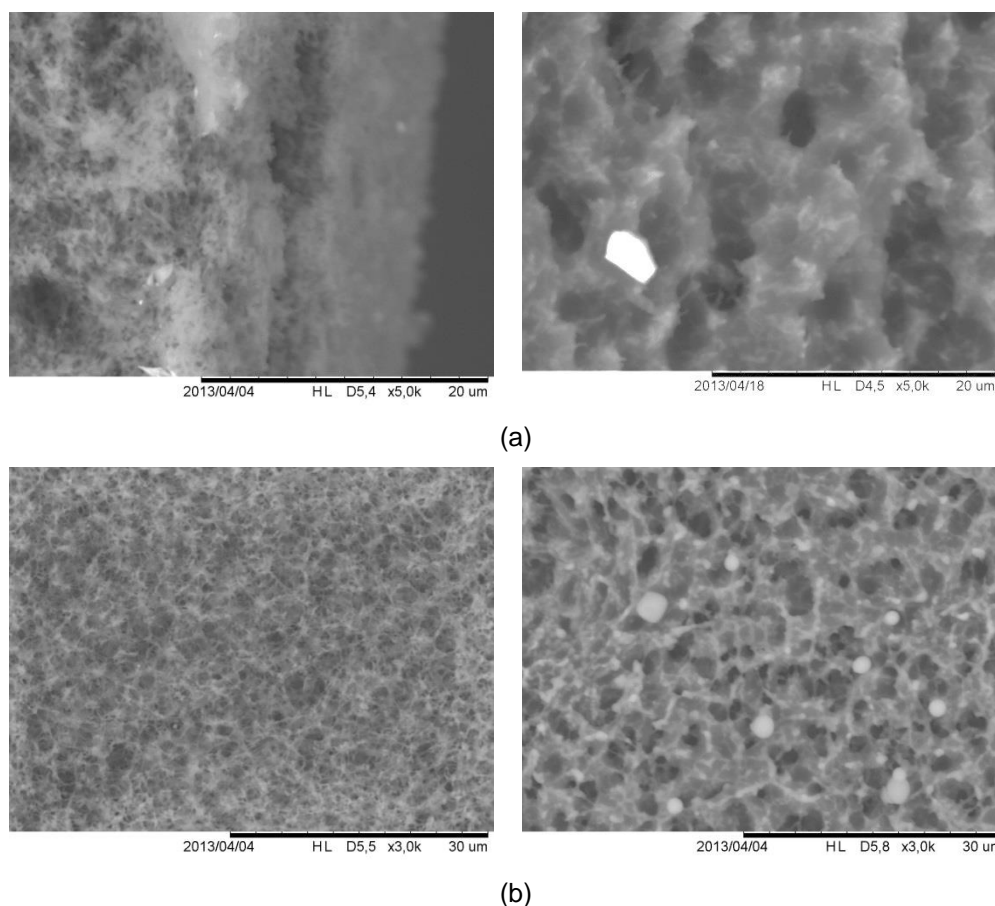


Figure 17. Transversal (a) and frontal (b) SEM images of new (on the left) and used (on the right) cellulose acetate membrane with mean pore size of 0.45 μm .

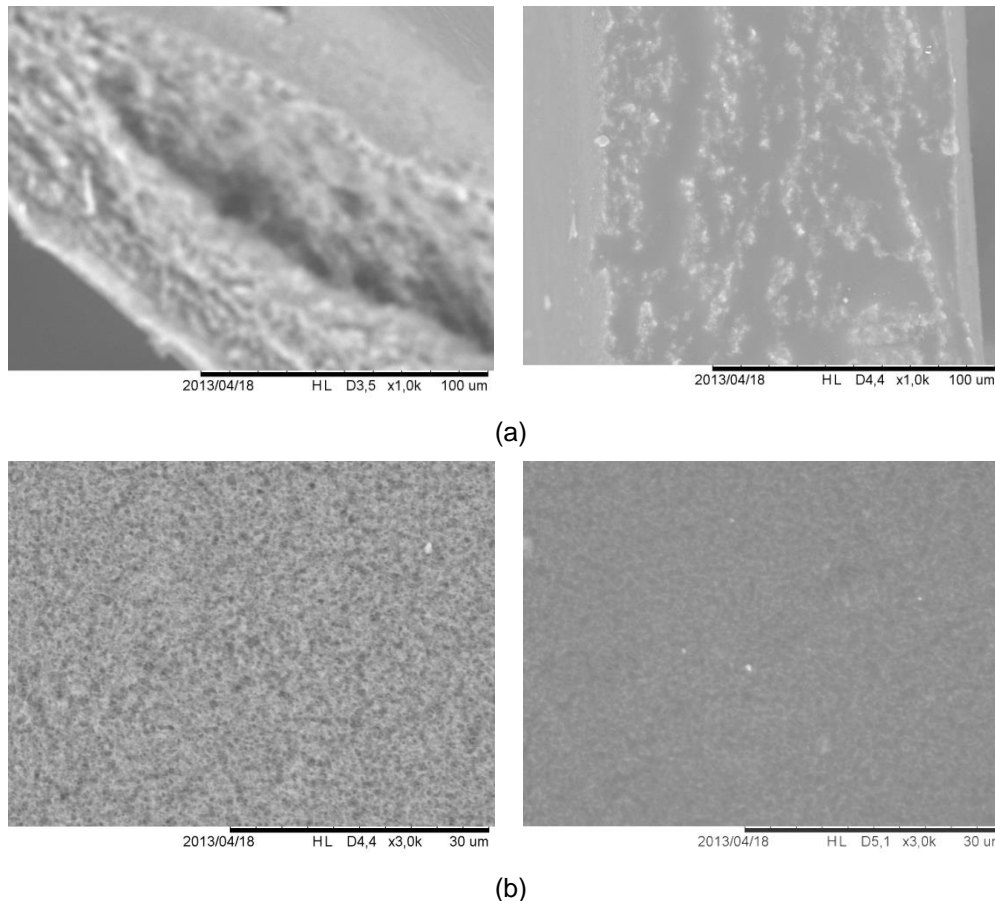


Figure 18. Transversal (a) and frontal (b) SEM images of new (on the left) and used (on the right) cellulose nitrate membrane with mean pore size of 0.22 μm .

In all the images obtained, it is possible to observe that besides the fouling phenomenon, there was a modification on the membrane surface, reinforcing the theory that the membrane is probably interacting with some component of the emulsion, probably the surfactant, and destabilizing the emulsion.

The presence of surfactants in water-in-oil emulsions has been widely identified as being responsible for the stability of these emulsions, due to electrostatic and hydrophobic interactions that “protect” droplets, and thus, the absence of this component may result in oil droplets coalescence. According to Del Colle *et al.* (2007), demulsification processes have a large influence of surface phenomena by strong physicochemical interactions between particles of mixture in processing and the membrane. Hlavacek (1995) observed that flux decline with time is attributed to surfactant adsorption on the pore walls, to the build-up of a polarized layer of concentrated emulsion at the membrane surface and also pore plugging by oil droplets, leading to demulsification.

As the problem was not detected to be the surfactant, since both presented the same behavior and the surfactant concentration was probably adequate, since 2% is the maximum concentration found in the literature, the phase separation observed in these tests was assumed to have occurred due to an adsorption of the surfactant onto the membrane, leading to droplets coalescence. Vladisavljevic *et al.* (2012) reported that when surfactant molecules adsorb to the membrane surface,

the membrane can become fouled by the surfactant molecules and the dispersed phase can spread over the membrane surface. This behaviour was observed by the same author when promoting water in oil emulsification with polypropylene hollow fibers (Vladisavljevic *et al.*, 2002). The authors observed indications of wettability changes and verified that pre-wetting the membrane with the continuous phase reduced the droplet size. For that reason, another test using the surfactant tween 20 was carried out applying the same conditions of concentration and pressure as in the test CAT20, however with previous treatment of the membrane before usage with an aqueous solution of the surfactant in order to saturate the membrane surface with the surfactant before using it and thus, avoid the adsorption. This process was called CAT20B. The behavior of the flux against time is shown in Figure 19.

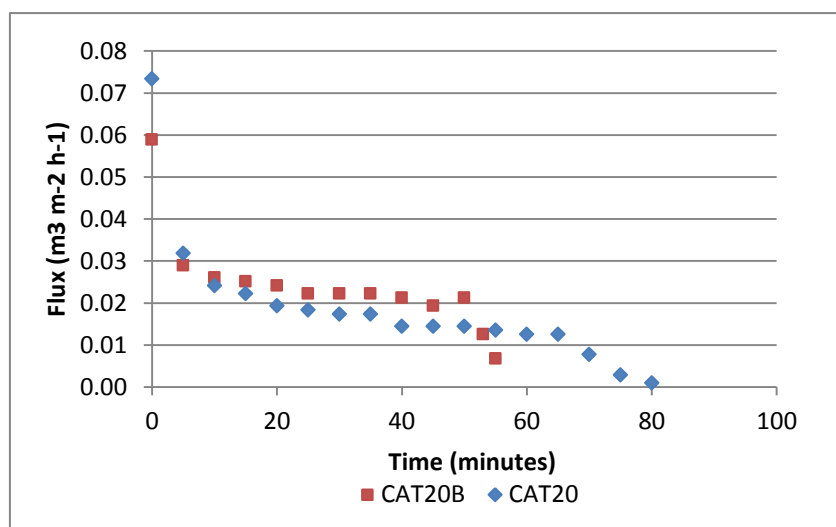


Figure 19. Flux of emulsion using cellulose acetate and Tween 20 as surfactant with (CAT20B) and without (CAT20) pretreatment with surfactant solution.

As it can be seen in the graph, the behavior of the emulsion was very similar with and without the pretreatment with aqueous solution containing surfactant, having a slightly better flux than the previous test, however with the same sharp flux decline on the beginning, indicating some kind of interaction and fouling. This result may lead to two different interpretations: (a) the interactions are not occurring on the membrane surface, but probably on the sintered plate; and (b) the immersion was not enough to saturate the membrane with the surfactant, so higher concentrations of the immersion bath for longer time has to be tested in the future.

It is also important to consider that, in spite of most of the membrane emulsification processes studied used this surfactant (Tween), for this membrane material and these pore sizes, some kind of interactions may occur and may not be overcome. Shima *et al.* (2004) used this type of membrane with the same pore size to produce double emulsions, however using SY-GLYS- TERw ML-750 (decaglycerol monolaurate) as the surfactant.

This kind of demulsification process using membrane technology was also widely studied (Lipp *et al.*, 1988; Hlavacek, 1995; Hoffmann & Nitsch, 1999; Kocherginsky *et al.*, 2003; Zhou *et al.*, 2010). According to Chakrabarty *et al.* (2010) this process is efficient for oily water treatment, since

the porous membrane matrix can promote coalescence of micron and submicron oil droplets into larger ones that can be easily separated by gravity.

One of the alternatives to overcome the fouling phenomenon and consequently to decrease the interactions between the emulsion and the membrane is to apply a shear stress on the membrane surface. In order to understand the influence of shear stress, a process with the same conditions as CAT20 (cellulose acetate membrane with mean pore size of 0.45 μm , 2% of surfactant concentration Tween 20 and 1 bar of pressure) was carried out, but this time with the agitated module (Figure 8), which promotes shear stress by magnetic agitation. This new process was called ACAT20.

Additionally, the influence of mean pore size was also studied. A process with the same type of material (mixed cellulose ester) and same process conditions but with mean pore size of 0.8 μm was carried out and called AMCT20. The flux along time of these two processes with agitation, as well as the process CAT20 are shown in Figure 20.

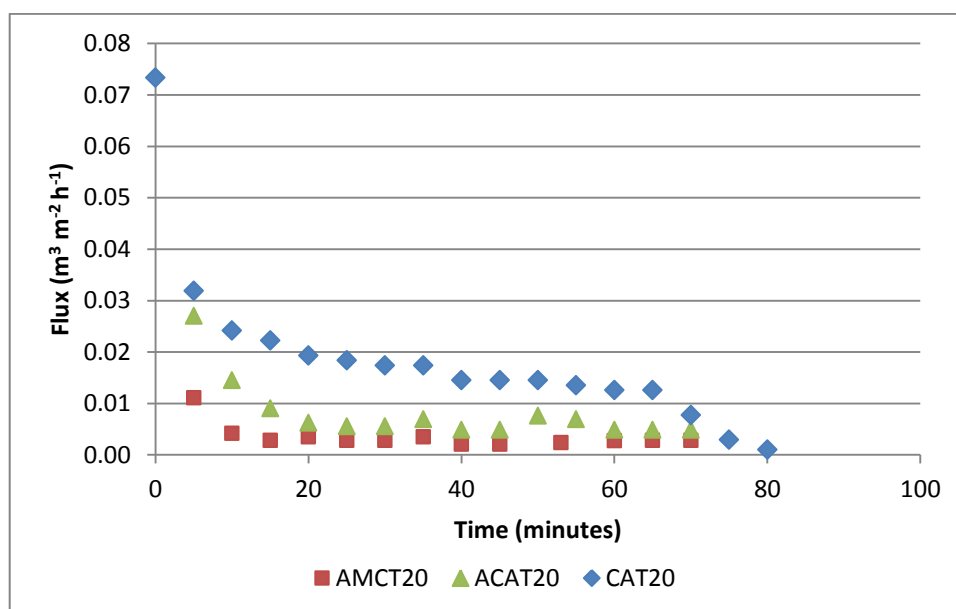


Figure 20. Comparison between the permeate fluxes obtained with agitated and non-agitated module.

It was possible to observe that the shear stress did not have a positive influence on the permeate flux, since both agitated processes showed a lower flux along time, even the process AMCT20 having greater pore size. However, this difference could be also due to the influence of the module design, since the modules presented different construction that may have influenced the permeation. As in the agitated module the sealing is mainly made by an o-ring, there is a possibility of gas escaping, and consequently loss of pressure.

The immediate stability of emulsions formed by these two additional processes was also evaluated and compared to the previous results. The agitation did not have influence on the emulsion stability, since both processes using the cellulose acetate membrane with pore size of 0.45 μm promoted demulsification. However, on the process ACAT20 the demulsification occurred by means of concentration instead of phase separation observed on CAT20. In order to try to better understand this behavior, another process was carried out with the same membrane, but this time applying a pressure

of 2 bar. Once more, the emulsion was not obtained, however the permeate collected presented this time phase separation. According to Li *et al.* (2009), coalescence within the pores depended on the applied pressure, being the emulsifications based on the ability of the transmembrane pressure to force the dispersed phase to pass through a membrane into the permeate.

Based on this, it was possible to observe that high pressures lead to phase separation, because the dispersed droplets are deformable and can be squeezed through the pores, causing coalescence. On the other hand, low pressures leads to emulsion concentration, since there is not enough pressure to make the droplets to pass through the membrane pores.

The shear stress showed to not have influence on emulsion stability, however, the pore size showed to be one of the most important parameters for emulsion production, since the process using the mixed cellulose ester membrane with greater pore size (0.8 μm) showed to be suitable for emulsion production. With an applied transmembrane pressure of 1 bar it was possible to obtain the stable emulsion on the permeate stream, without phase separation or concentration, with an average flux of 0.003 $\text{m}^3 \text{m}^{-2} \text{h}^{-1}$. The photomicrograph of the coarse emulsion and the emulsion achieved by the process AMCT20 obtained by optical microscope with magnification of 400 times is shown in Figure 21.

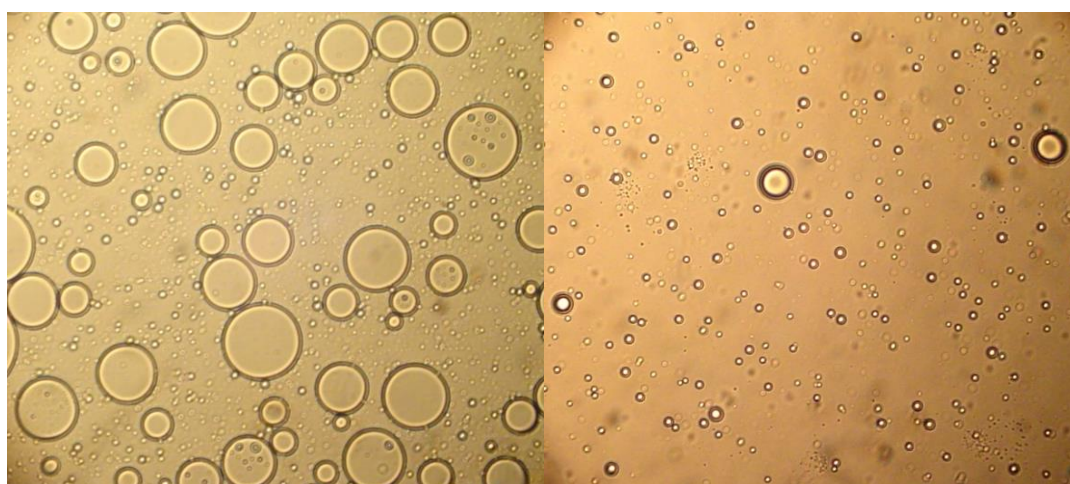


Figure 21. Photomicrograph of coarse emulsion (left) and emulsion achieved by process AMCT20 (right).

All the results obtained for agitated cell are summarized in Table 4.

Table 4 . Results obtained for premix membrane emulsification performed with agitated module.

Test	Oil	Membrane pore size (μm)	Pressure (bar)	Flux ($\text{m}^3 \text{m}^{-2} \text{h}^{-1}$)	Emulsion Formation
ACAT20	Sunflower	0.45	1.0	0.008	No (concentration)
ACA2T20	Sunflower	0.45	2.0	0.027	No (coalescence)
AMCT20	Sunflower	0.80	1.0	0.003	Yes

Once the production of sunflower oil emulsion using the agitated module with mixed cellulose ester membrane with pore size of 0.8 μm was possible, a test with linseed oil on the same process

conditions was carried out. This process was called PME 1. Average flux, stability and microscopy were evaluated.

As for sunflower oil, it was possible to obtain stable emulsion using 1 bar of pressure. The flux was similar to sunflower oil with an average value of $0.009 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$. The stability for 7 days was measured by means of % of phase separation and the data was fitted on a logarithmic behavior (Figure 22).

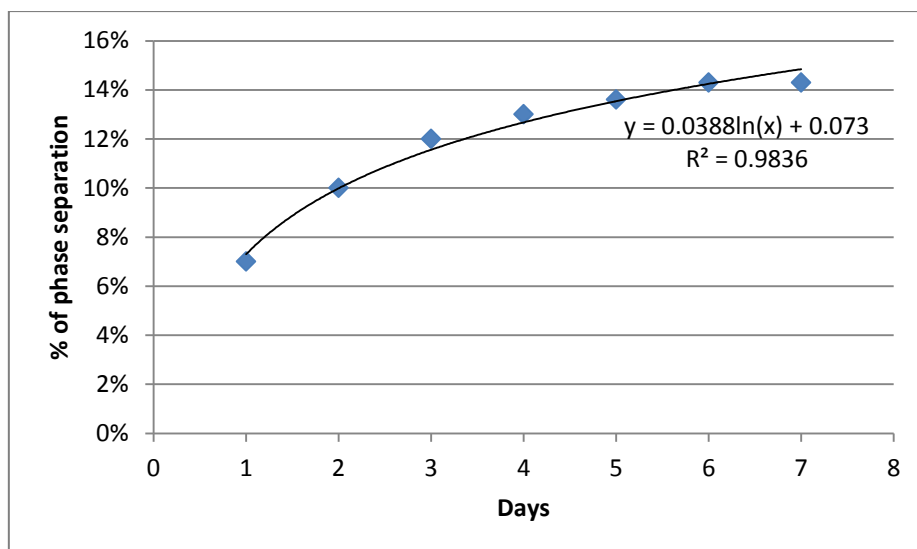


Figure 22. Stability of linseed oil emulsion prepared by PME 1.

In order to evaluate the influence of pressure on emulsion formation, two more tests were done with pressures of 2 and 3 bars and called PME 2 and PME 3, respectively, in order to compare with PME 1. Average flux, microscopy, particle size distribution and capacity of emulsion formation were evaluated.

All of the pressures applied showed to be suitable for emulsion production, since in all the cases, stable emulsions were produced. From the fluxes obtained with the three applied pressures, it was possible to obtain the permeability for the emulsion production. The average flux of all the processes are shown in Figure 23.

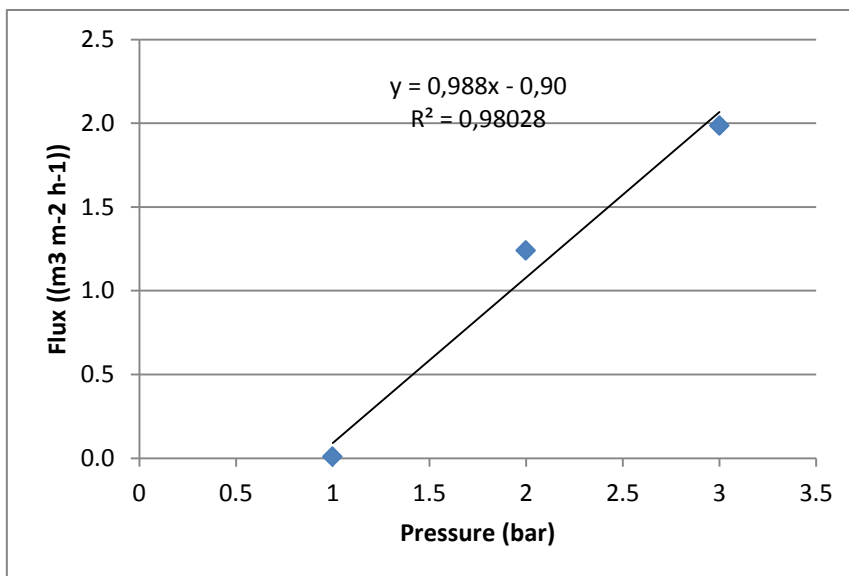


Figure 23. Flux against pressure for processes PME1, PME 2, PME 3.

Applying Darcy's law (Equation 3) to the equation obtained in Figure 23, it was possible to obtain an emulsion permeability of $0.988 \text{ m}^3 \text{ h}^{-1} \text{ m}^{-2} \text{ bar}$. Besides, a minimum pressure of 0.90 bar has to be applied to emulsion formation though mixed ester cellulose membrane with mean pore size of $0.8 \mu\text{m}$.

The particle size distribution was analyzed by microscopy and by laser diffraction, and it is shown in Figures 24 and 25, respectively.

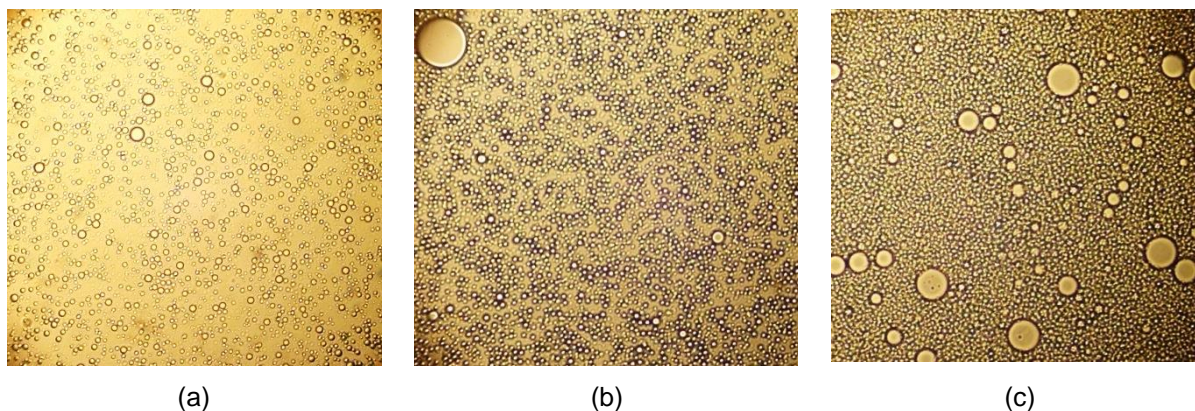


Figure 24. Photomicrograph with 400x magnification of emulsion prepared by PME 1 (a), PME 2 (b) and PME 3 (c).

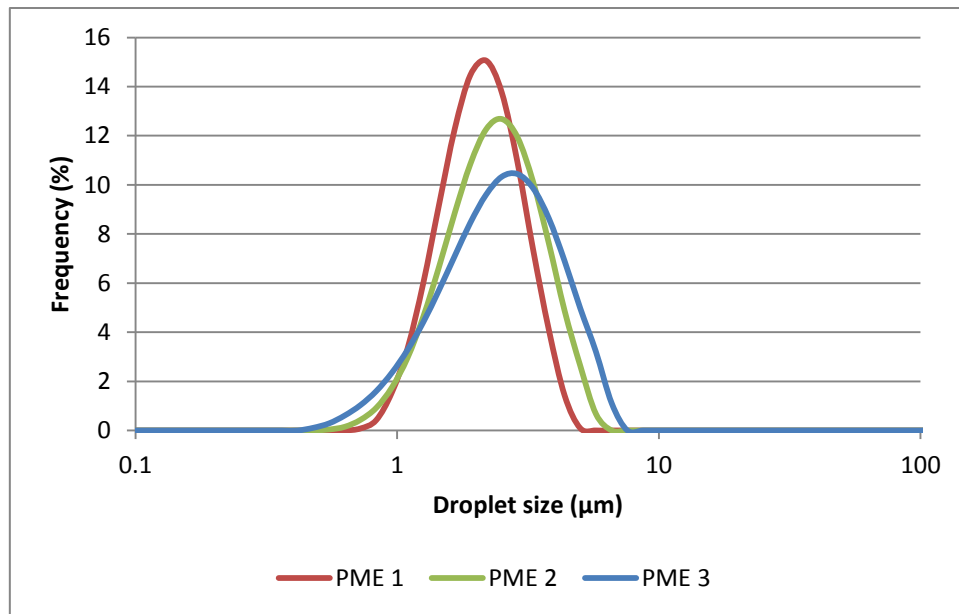


Figure 25. Droplet size distribution of processes PME 1, PME 2 and PME 3.

According to Figures 24 and 25, the increase on pressure caused, besides the increase on flux, also an increase on droplet size. It was also possible to observe that the distribution was narrower when lower pressure was applied. This results can be confirmed by the values of surface weighted mean diameter ($D[3,2]$), De Brouckere mean diameter and span showed in Table 5.

Table 5 . Surface weighted ($D[3,2]$), volume weighted $D[4,3]$ and span values for processes PME 1, PME 2 and PME 3.

Process	Pressure (bar)	$D[3.2]$ (μm)	$D[4.3]$ (μm)	Span
PME 1	1.0	2.19	2.27	1.10
PME 2	2.0	2.26	2.76	1.27
PME 3	3.0	2.26	2.93	1.34

Membranes with greater pores were not tested, since the droplet size achieved would be probably larger, which would be undesirable, since one of the greatest advantages of emulsion production by membrane emulsification is the small droplet size.

In order to improve monodispersity of the linseed emulsion, multi-stage premix emulsification was carried out. Taking into account the flux and the droplet size distribution, the process PME 2 was chosen to execute cycles and to evaluate the performance and the emulsion behavior by means of droplet size distribution and flux during each cycle. The tests were called PMEC followed by the number of cycles, being the PMEC.0 the inlet feed prepared on the Ultra-Turrax blender. The results are summarized on Table 6.

Table 6 . Flux and droplet size parameters for the process PME 2 accomplished on cycles

Tests	Flux ($\text{m}^3 \text{m}^{-2} \text{h}^{-1}$)	D[3.2] (μm)	D[4.3] (μm)	Span
PMEC.0	0.00	3.39	8.85	2.30
PMEC.1	1.35	2.26	2.93	1.35
PMEC.2	1.71	2.07	2.33	0.92
PMEC.3	2.54	1.98	2.12	0.68
PMEC.4	3.32	1.89	2.02	0.68
PMEC.5	3.40	1.80	1.92	0.67

From the results obtained it was possible to observe that droplet size decreased along the number of cycles, by means of the two parameters that measures the diameter in terms of surface and volume. From the coarse emulsion prepared in the Ultra-Turrax equipment to the emulsion obtained after the fifth cycle, there was a decrease on Sauter mean diameter from 3.885 to 1.801 μm and even a bigger influence was observed on the De Brouckere mean diameter since it decreased from 8.847 to 1.920 μm . This difference is explained by the fact that the De Brouckere mean diameter is more sensitive to the presence of large particulates in the size distribution (Malvern, 2012), and in this case, it represents better the droplet size of the coarse emulsion. Also a decrease on span values was observed, showing that the droplet size distribution is was getting narrower along the cycles, going decreasing from 2.301 to 0.671. However, none of them could be considered as monodisperse, since for that, span values obtained has to be lower than 0.4 (Nazir *et al.*, 2010).

As reported before by several authors (Vladislavjevic *et al.*, 2004b; Henelyta *et al.*, 2005; Zhou *et al.*, 2008; Trentin *et al.*, 2010), there was a flux increase along the cycles. According to Nazir *et al.* (2010), this effect is most probably a result of the decreased viscosity related to droplet size reduction. Besides, if the droplet size is more similar to the pore size, it is expected to pass unhindered, and less pressure is needed. Moreover, this is also an indication that low or even no fouling is occurring on this membrane, since the same membrane is used for all the cycles without any cleaning procedure.

In order to better visualize these results, the particle size distribution in each cycle is shown in Figure 26.

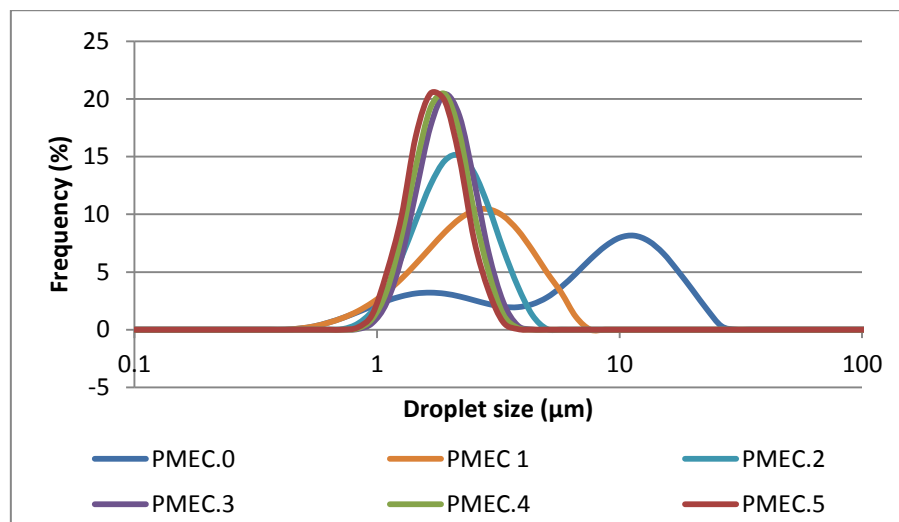


Figure 26. Particle size distribution of the process PME 2 accomplished on cycles.

The coarse emulsion, even not presenting very larger Sauter diameter than in the cycles, showed to have a droplet size distribution much wider, having a bimodal distribution, which was not observed for emulsions prepared by membrane emulsification, even in the first cycle. Besides, it was possible to observe that after the third cycle, the influence of cycles was not so significant, and the droplet size distribution showed very similar behavior for cycles 3, 4 and 5.

4.2 Direct Membrane Emulsification (DME)

For all the experiments done in the experimental design proposed in Table 3, the hydraulic permeability was measured before each test. The graph showing the variation of this parameter is shown in Figure 27.

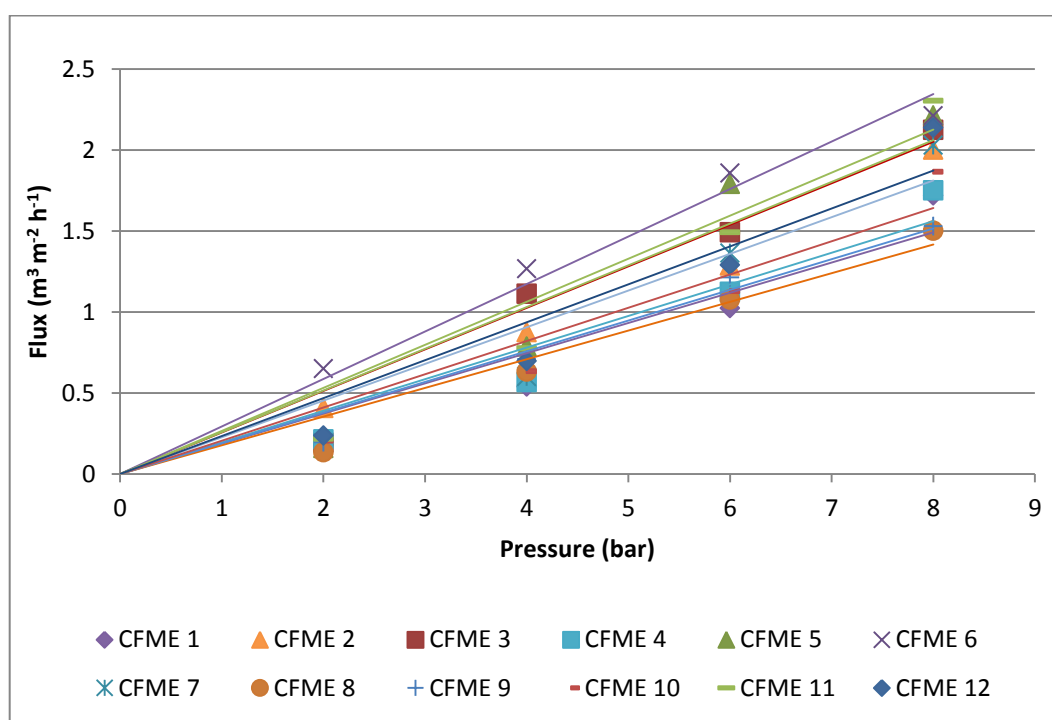


Figure 27. Hydraulic permeability of ceramic membrane with mean pore size of 0.2 μ m.

A good reproduction of permeate flux was observed, showing that the cleaning procedure established was effective. From Darcy's law (Equation 3) and the equations obtained for each curve, it was possible to calculate the hydraulic permeability and the membrane resistance,

The average value for hydraulic permeability were $0.24 \pm 0.06 \text{ m}^3 \text{ h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$ and the membrane resistance found was $6.67 \times 10^{-13} \text{ m}^{-1}$. This resistance can be considered very low, once Vladisavljevic *et al.* (2004a) found for membrane with mean pore size of 0.5 μm and very similar porosity and thickness (0.35 and 20-30 μm respectively) the value of approximately $1 \times 10^{-11} \text{ m}^{-1}$.

The processes were evaluated by means of flux, droplet size distribution (by optical microscopy and laser diffraction) and its parameters ($D[3.2]$, $D[4.3]$ and span) and stability (by % of phase separation and optical microscopy for 14 days). All the results are summarized in Table 7.

Table 7 . Flux, droplet size and stability results for linseed oil cross flow membrane emulsification

	Pressure (bar)	Velocity (m s ⁻¹)	Tween concentration (%)	Flux (m ³ m ⁻² h ⁻¹)	D [3.2] (μm)	D [4.3] (μm)	Span	Stability for 14 days (% of separation)
CFME 1	4.5	3.0	3.0	0.08	1.44	1.66	1.01	8.5
CFME 2	4.5	3.0	1.0	0.08	1.77	2.09	1.12	13.6
CFME 3	3.0	5.5	2.0	0.02	1.53	1.78	1.06	10.0
CFME 4	3.0	5.5	2.0	0.07	1.12	1.26	0.95	6.0
CFME 5	1.5	3.0	1.0	0.01	1.14	1.34	1.12	6.9
CFME 6	1.5	8.0	3.0	0.12	1.43	1.63	0.97	7.5
CFME 7	3.0	5.5	2.0	0.06	0.99	1.09	0.81	4.0
CFME 8	1.5	8.0	1.0	0.04	0.90	1.03	1.03	8.2
CFME 9	4.5	8.0	1.0	0.11	1.22	1.38	0.98	8.0
CFME 10	4.5	8.0	3.0	0.12	1.30	1.69	1.57	7.0
CFME 11	1.5	3.0	3.0	0.02	1.23	1.36	0.87	6.0
CFME 12	3.0	5.5	2.0	0.07	1.47	1.76	1.18	9.6

From these results, statistic evaluation was done in order to analyse which factors have greatest influence on the dispersed phase flux. The Pareto chart is represented in Figure 28.

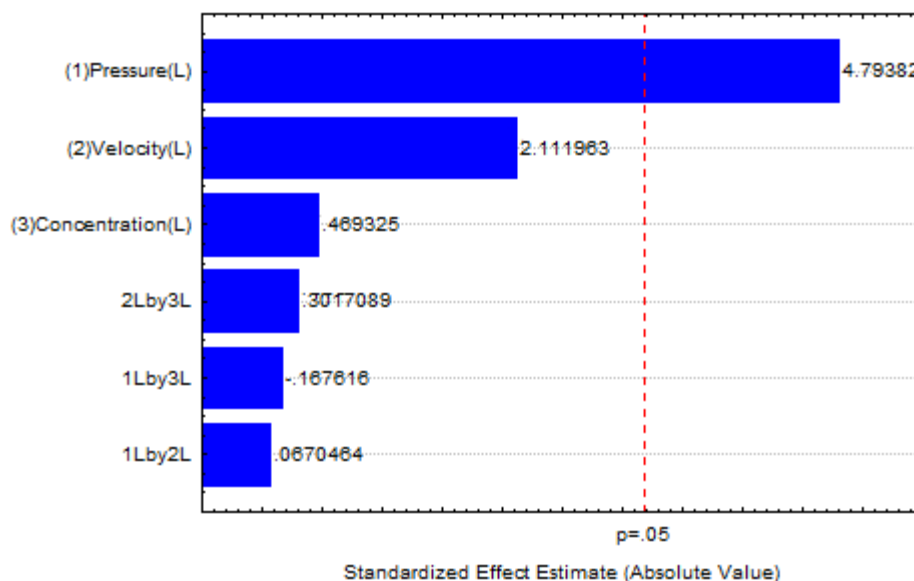


Figure 28. Pareto chart for the dispersed phase flux in cross flow membrane emulsification.

In a confidence interval of 95%, the transmembrane pressure was the only parameter that had a significant effect on the flux. From the positive value indicated by its influence, it is possible to infer that the increase of transmembrane pressure led to the increase of the dispersed phase flux. This result was expected, since the transmembrane pressure is the driving force of the process, and for that reason is directly related to the transmembrane flux. The other parameters did not have a significant effect

In order to predict a model for this dependent variable (flux), non-significant factors were excluded and the significance of regression and lack of fit were verified in relation to a confidence interval of 95% ($p \leq 0,05$) through F variance test (ANOVA). Table 8 presents the calculated and tabulated (probability) F value.

Table 8 . ANOVA analysis of adjusted model for flux.

	SS	df	MS	F _{calculated}	F _{probability*}
Regression	0.010	1	0.0102	27.046	4.256
Residue:	0.004	10	0.0004		
-Lack of fit	0.002	7	0.0003	1.863	8.941
-Pure error	0.001	3	0.0004		
Total SS	0.014	11			

*F tabulated values at $p \leq 0,05$.

SS = Sum of squares, df = degree of freedom, MS = Mean squares

The codified model to represent the flux resulting from cross flow emulsification of linseed oil within the boundaries established for cross flow velocity, surfactant concentration and transmembrane pressure is described by the Equation 12.

$$\text{Flux} = 0.060 + 0.036P \quad (12)$$

Where P is the transmembrane pressure.

The determination coefficient (R^2) for the adjusted model was 0.7301, indicating that the model explained 73.01% of the obtained data variation.

The adjusted model obtained for flux in the process of cross flow membrane emulsification of linseed oil showed to be predictive. This can be affirmed based on Table 8, since the model presented significant regression in the confidence level of 95% ($F_{\text{calculated}}$ greater than $F_{\text{probability}}$) and the lack of fit was not significant in the same confidence level ($F_{\text{calculated}}$ smaller than $F_{\text{probability}}$).

A linear behavior could be observed for pressure influence on flux, since none of the other independent variables had significant influence on this response.

Particle size and its distribution were evaluated by means of laser diffraction. The results are shown in Figure 29, where central points are highlighted with dashed lines.

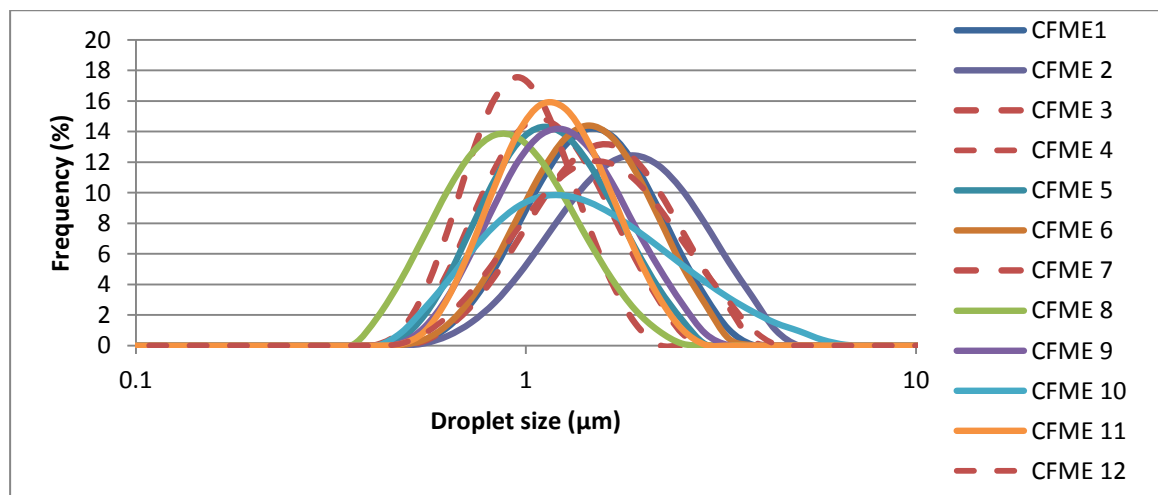


Figure 29. Droplet size distribution of linseed oil emulsions produced by cross flow membrane emulsification.

From Figure 29 it was not possible to observe a clear influence of process parameters on droplet size and its distribution, since all the process had similar behavior. In order to try to find a model for the droplet size behavior, statistic analysis were carried out for the two parameters that evaluate the droplet size ($D[3.2]$ and $D[4.3]$) and the parameter that evaluates the droplets distribution on the sample (span). Figures 30 and 31 represent the Pareto chart of effects for the surface weighted mean diameter ($D[3.2]$) and volume weighted mean diameter ($D[4.3]$) respectively.

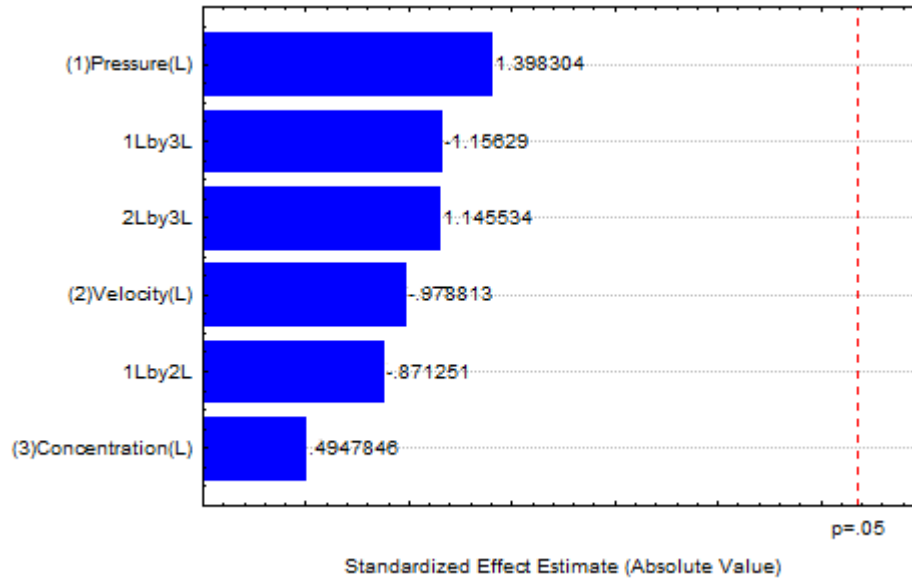


Figure 30. Pareto chart for surface weighted mean diameter in cross flow membrane emulsification

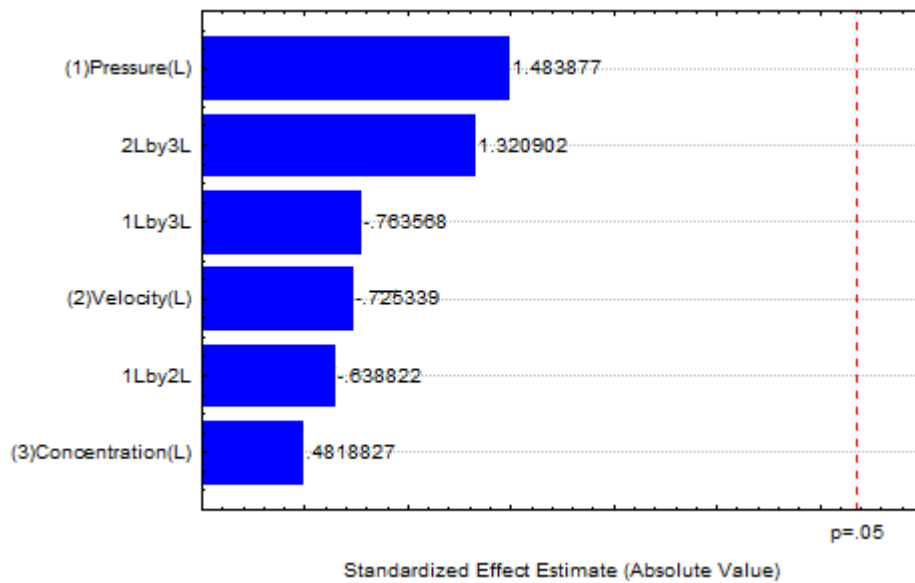


Figure 31. Pareto chart for volume weighted mean diameter in cross flow membrane emulsification

Sauter mean diameter ($D[3.2]$) and De Brouckere mean diameter ($D[4.3]$) results showed similar behavior. Results showed that there was no significant difference on both parameters that represents the droplet size within the range chosen for the studied variables.

Schröder *et al.* (1998) have shown that the droplet size depends on the type of surfactant or rather the adsorption kinetics on the formation times of the droplets. If the formation time is long compared to the time of decreasing interfacial tension, then the interfacial tension dynamics have little or no influence on the droplet size and the size remains unchanged on increasing flux. As it was shown before, the increase on pressure leads to an increase on flux. Thus, the droplet size remains unchanged on increasing pressures.

Usually, the droplet size decreases sharply as the crossflow velocity increases however, at one point, it reaches a size where it becomes independent on the flow velocity. Thus, more pronounced changes in droplet size occur at small shear stresses (Jocelyne & Trägårdh, 2000). So, probably the range of velocities used in the experimental design was higher than the point at which velocity has a great influence on the droplet size. Besides, Williams *et al.* (1998) already observed no influence of cross-flow velocity on droplet size for o/w emulsions up to 30% oil.

From Table 7 it was possible to observe that none of the tests produced monodisperse emulsions, since the lowest span achieved was 0.806 and to be considered monodisperse, this number has to be below 0.4. However, similar values were obtained by Williams *et al.* (1998), which obtained the span of 0.83 using an α -Al₂O₃ membrane with the mean pore size of 0.5 μ m.

This result could be explained by the α -alumina membrane morphology, since this largely determines the obtained average droplet size and the droplet size distribution. The used membrane has very low membrane resistance and does not have a narrow pore size distribution. According to Gijssbertsen-Abrahamse *et al.* (2004), in general, low total membrane resistance promotes large disperse phase flux, however, to obtain monodisperse emulsions the membrane resistance should be high. Besides, the manufacturing method used for the production of α -Al₂O₃ membranes promotes pore interconnectivity enabling coalescence on membrane surface. Moreover, the module to achieve direct emulsification requires cross flow, and thus, involves a pump that can break the droplets, modifying the droplet size formed on the membrane surface.

The droplet size distribution was evaluated also by statistic analysis in terms of span, as shown in Figure 32.

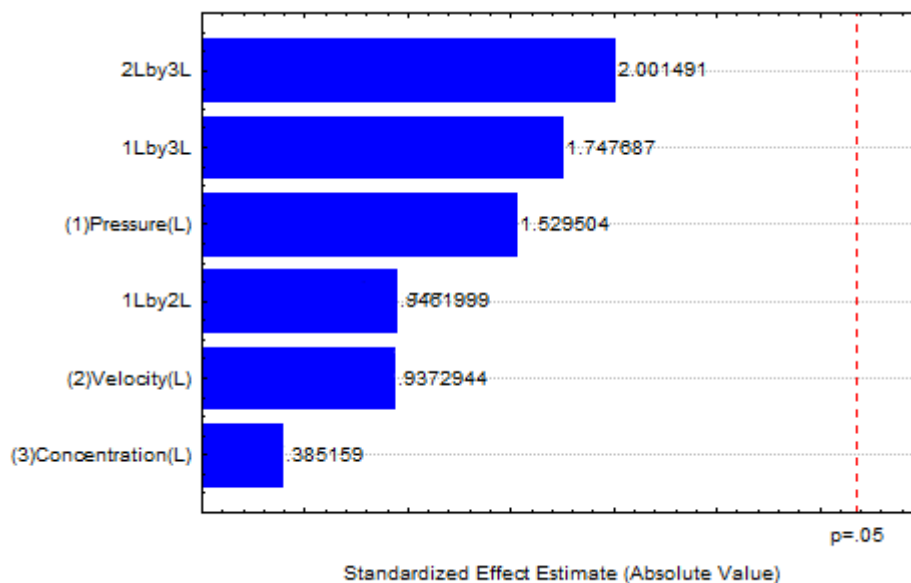


Figure 32. Pareto chart for span in cross flow membrane emulsification

As for Sauter and De Brouckere diameter, there was no significant difference on span within the range chosen for the studied variables.

Besides the droplet size, another very important parameter is the emulsion stability. This is required for food products since is one of the parameters that determines the product shelf life. The stability was evaluated in terms of % of separation and microscopy. The results of % of separation along time for all the trials are shown in Figure 33.

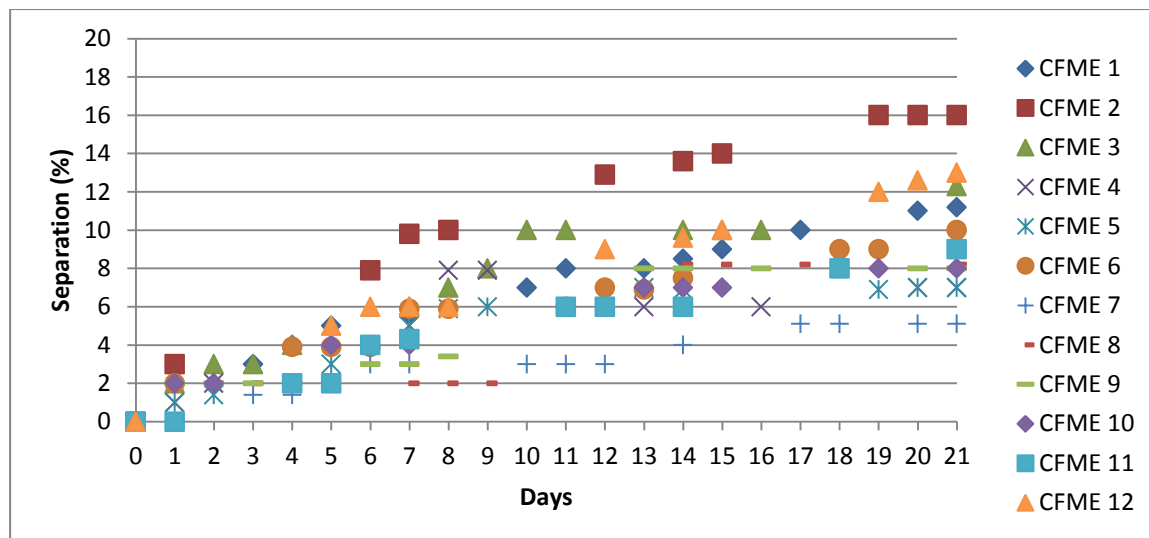


Figure 33. Separation of linseed oil cross flow membrane emulsification

It was possible to observe that the trial which presented lower stability (represented by higher cream formation (% of separation)) was the CFME 2, where the highest transmembrane pressure and the lowest cross flow velocity and surfactant concentration were applied. This result is in agreement with the Pareto chart (Figure 34), even the effects showing to not be significant. .

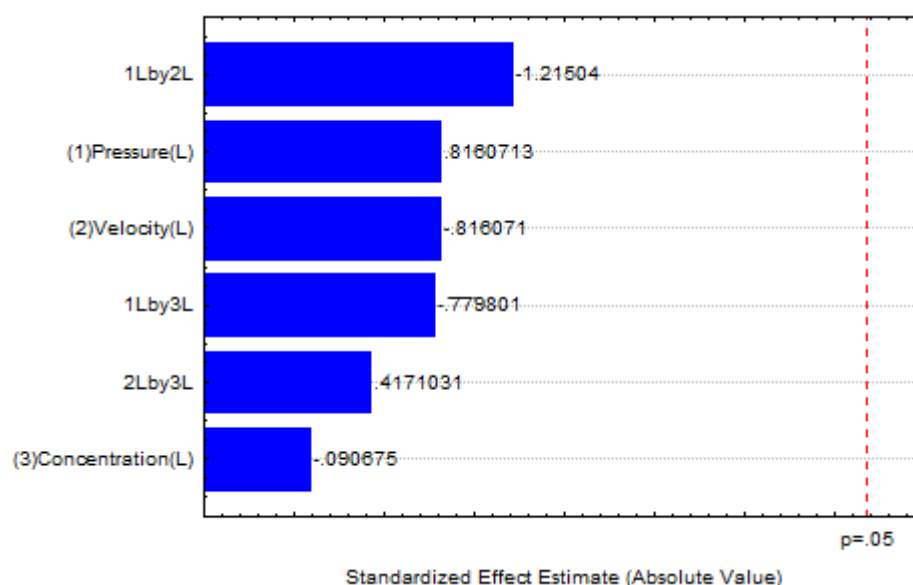
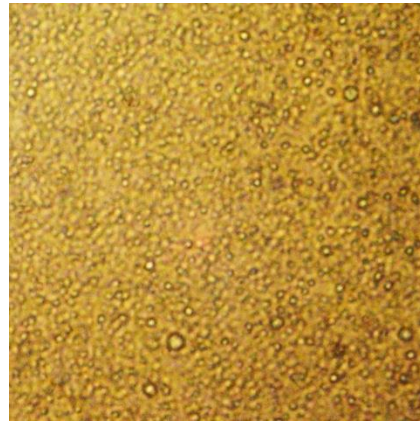
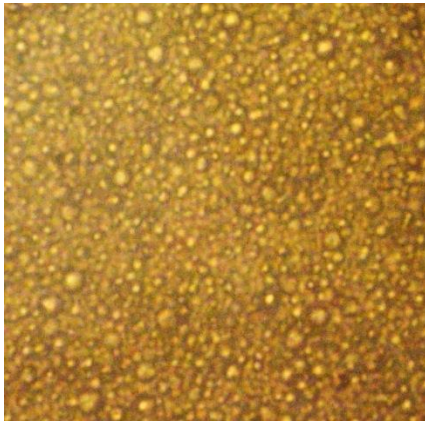


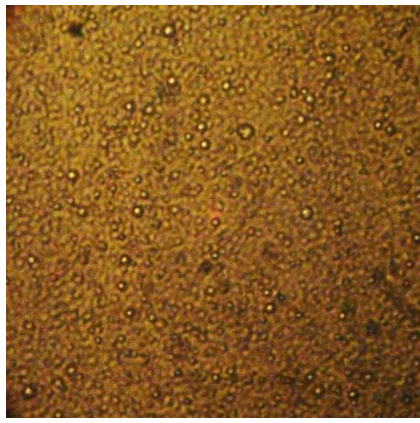
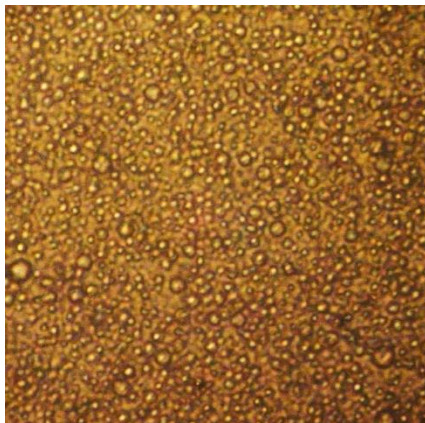
Figure 34. Pareto chart for % of phase separation in cross flow membrane emulsification

As for particle size, none of the parameter evaluated showed significant difference within the range of variable studied for phase separation.

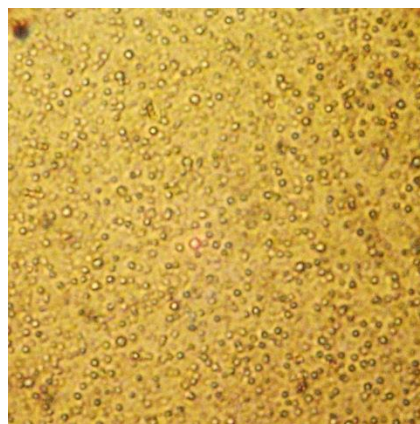
The stability was also evaluated by means of microscopy analisys. Photomicrographs with magnificatin of 1000 x were taken just after the emulsion preparation and after 21 days of the lower phase of the graduated cylinder (emulsion phase). The results are shown in Figure 41.



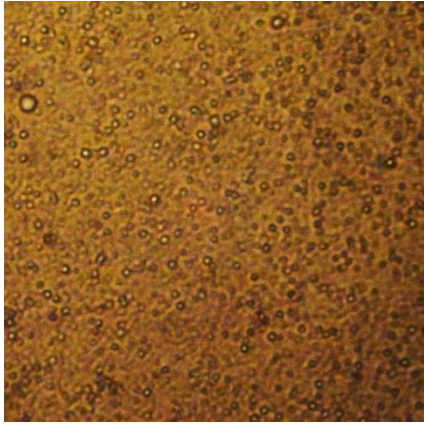
CFME 1



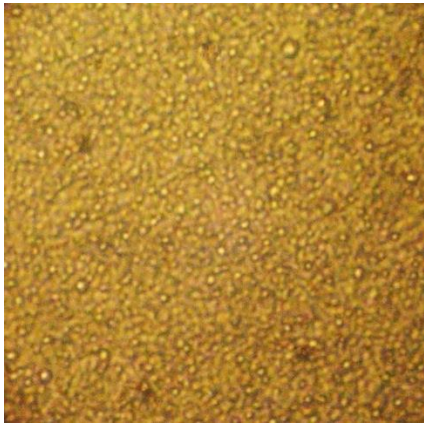
CFME 2



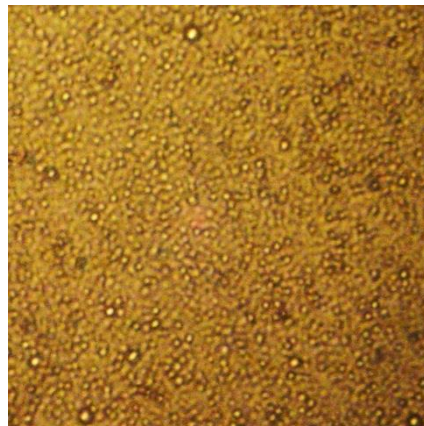
CFME 3



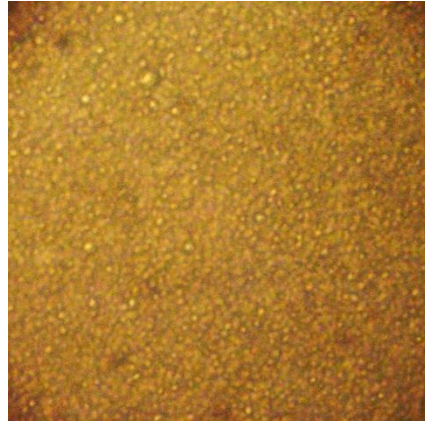
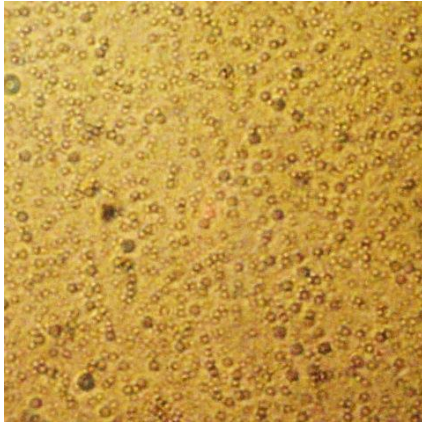
CFME 4



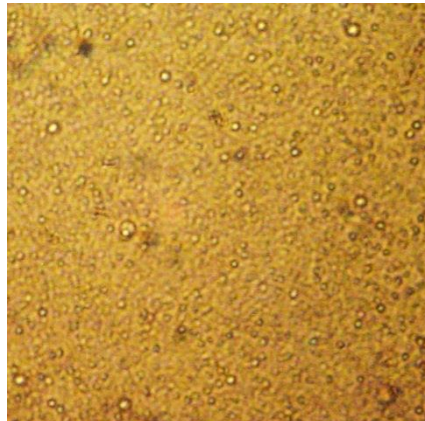
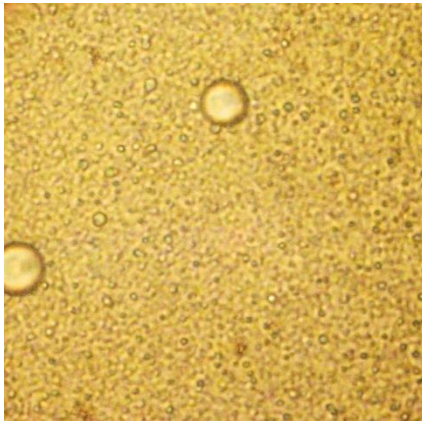
CFME 5



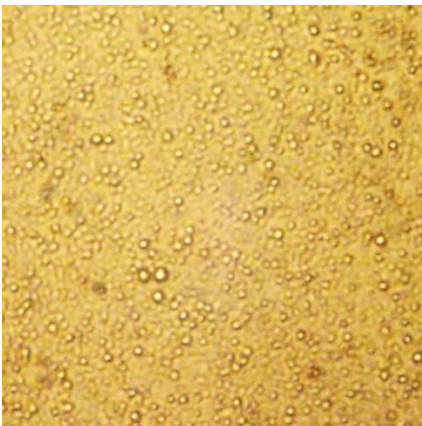
CFME 6



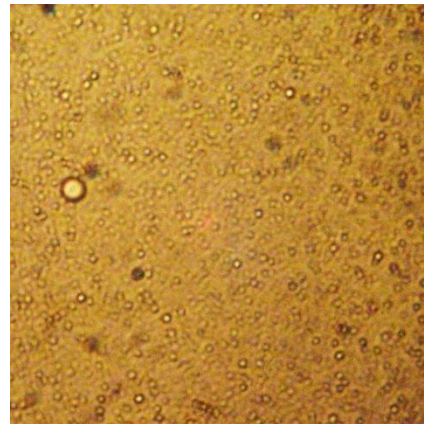
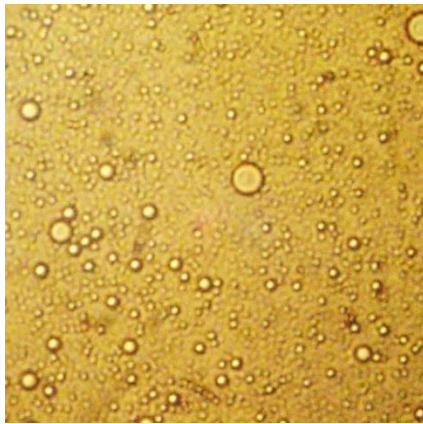
CFME 7



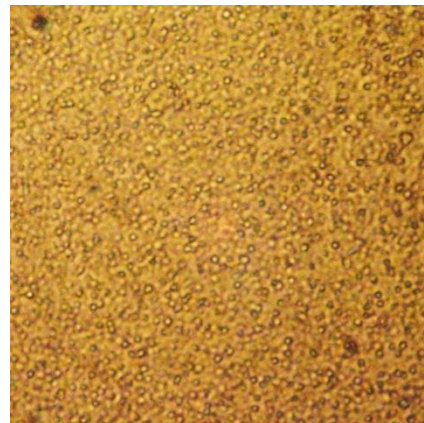
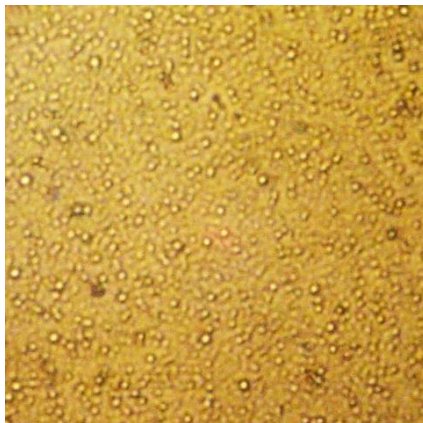
CFME 8



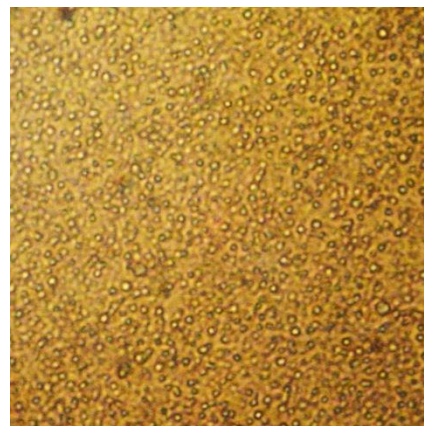
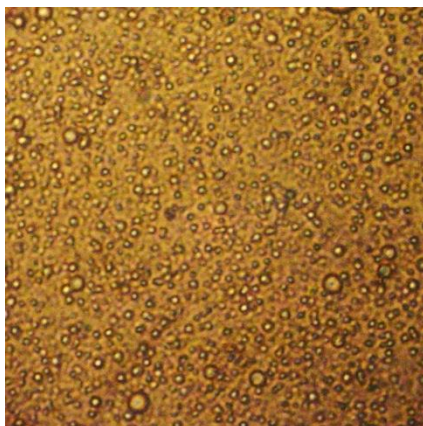
CFME 9



CFME 10



CFME 11



CFME 12

Figure 35. Photomicrographs of linseed oil cross flow membrane emulsification in the first day (left) and twenty-first (right)

It was possible to observe that although the cream formation and phase separation of all the emulsions formed, in 21 days, the oil droplet size was visually similar as in the first day.

4.3 Comparison between emulsification methods

An ideal comparison of the two different methods for the emulsions production by membrane emulsification would be producing both of them in the same module, using the same membrane with

the same conditions. So, trials were done in the tubular module with the ceramic membrane to produce emulsion by means of the premix method. The process was tested with four different pressures, from 3 to 6 bar, but all of them promoted demulsification.

In order to choose the most suitable process for emulsion production, the coarse emulsion produced in the Ultra-Turrax was compared to the emulsions produced by premix and direct (cross flow) membrane emulsification, even being different membrane materials with different pores size. The comparison was done in terms of emulsion flux, droplet size and its distribution and stability for 7 days.

For that comparison, Tween 20 was used at concentration of 2%, and a pressure of 3 bar were used in both types of membrane emulsification. For cross flow membrane emulsification, the central point velocity was used (5.5 m s^{-1}).

For the emulsion flux comparison, only the premix and direct membrane emulsification process were compared, once the production of coarse emulsion is done in batch and does not have inlet and outlet streams. Several authors suggest that one of the biggest advantages of premix membrane emulsification is the very high transmembrane flux when compared to direct ME. However, the transmembrane fluxes cannot be compared, once in premix ME, the permeate flux is the emulsion flux and on the other hand on direct ME, the transmembrane flux is the oil flux to form the emulsion. In order to standardize this parameter (once both have the same oil concentration), emulsion productivity were calculated in terms of emulsion volume per membrane unit area per time. For premix membrane emulsification, the productivity is equal to the transmembrane flux, however, for direct membrane emulsification productivity was calculated taking into account the total volume of emulsion produced and not only the transmembrane flux. The results are shown in Table 9.

Table 9 . Emulsion flux by Premix and direct ME

Type of ME	Volume of emulsion (L)	Membrane area (m^2)	Time (hours)	Flux ($\text{m}^3 \text{ m}^{-2} \text{ h}^{-1}$)
Direct	1.250	0.005	0.890	0.28
Premix	0.025	0.002	0.007	1.79

In terms of productivity, the premix showed to be more suitable for emulsion production, since it presented at 3 bars flux six times higher than direct ME. However, it is important to emphasize that the pore size of the membrane used for premix membrane emulsification was four times higher than the membrane used for direct membrane emulsification.

Figure 36 shows the droplet size and its distribution in terms of microscopy with magnification of 400 for the three processes.



(a) (b) (c)
Figure 36. Photomicrographs of Ultra-Turax emulsification (a), premix ME (b) and direct ME (c)

From the photomicrographs it is possible to observe the difference on the droplet size formed by different methods. Much smaller droplets with narrower distribution were produced by direct membrane emulsification than in the other two methods. However, it is possible to observe that premix ME also promotes the achievement of very small droplets, but with wide distribution. This results can be confirmed by Figure 43, where the laser diffraction results are expressed.

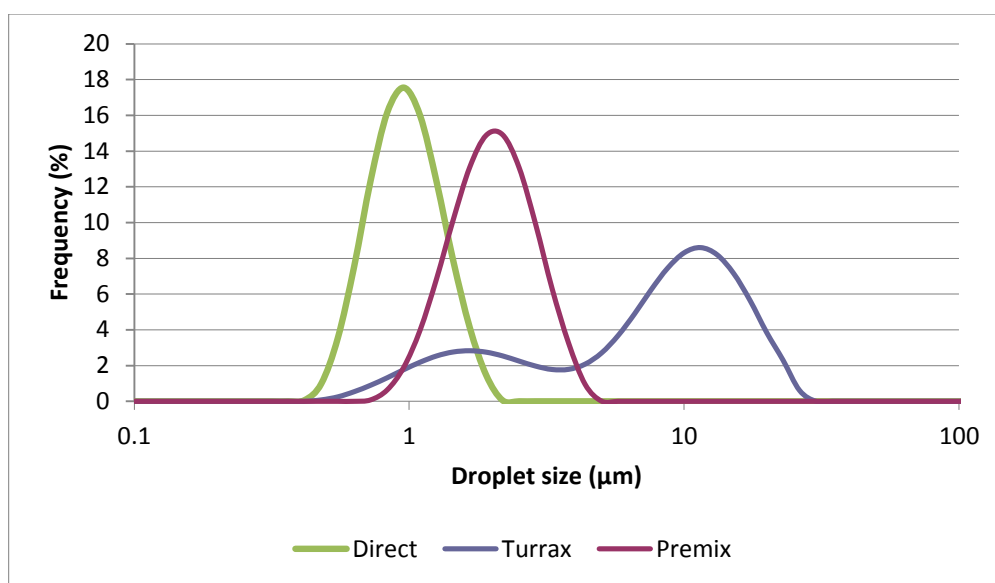


Figure 37. Droplet size distribution for emulsion achieved by Ultra-Turax, direct ME and premix ME

These results confirm the behavior observed in Figure 36, showing that to produce smaller and less polydisperse emulsion, the direct membrane emulsification was the most suitable. It is important to highlight again that this result probably has the influence of the difference in the membrane pore size. According to equation (1), droplet diameter is linearly dependent on the pore diameter. Thus in order to try to overcome this difference between the processes, and then make them comparable, the constant c (ratio between the droplet size and the pore size) was calculated. Results are shown in Table 10 together with the droplet size parameters.

Table 10 . Span, Sauter and De Brouckere mean diameter for emulsion achieved by Ultra-Turrax, direct ME and premix ME,

	D[3.2] (μm)	D[4.3] (μm)	Span	c
Direct ME	0.99	1.09	0.81	4.97
Premix ME	2.09	2.36	0.92	2.62
Turrax	3.73	8.87	1.94	

When we compare the droplet size by means of Sauter and De Brouckere mean diameter, the direct membrane emulsification seems to be more suitable, however, this is due to the smaller membrane pore size. For that reason, the constant c showed to be a useful parameter to compare the processes, since it shows the relationship between the droplet size and the membrane pore size. Taking this into account, once more the premix membrane emulsification showed better results, since the constant c was much smaller than for direct membrane emulsification.

To compare the stability of the processes, cream formation by means of % of separation was evaluated in 7 days. The separation were 29,0 %, 14,3% and 3,0% for the emulsions produced by Ultra-Turrax, premix ME and direct ME respectively. The photos of the graduated cylinder at day 7 for each method is shown in Figure 38.

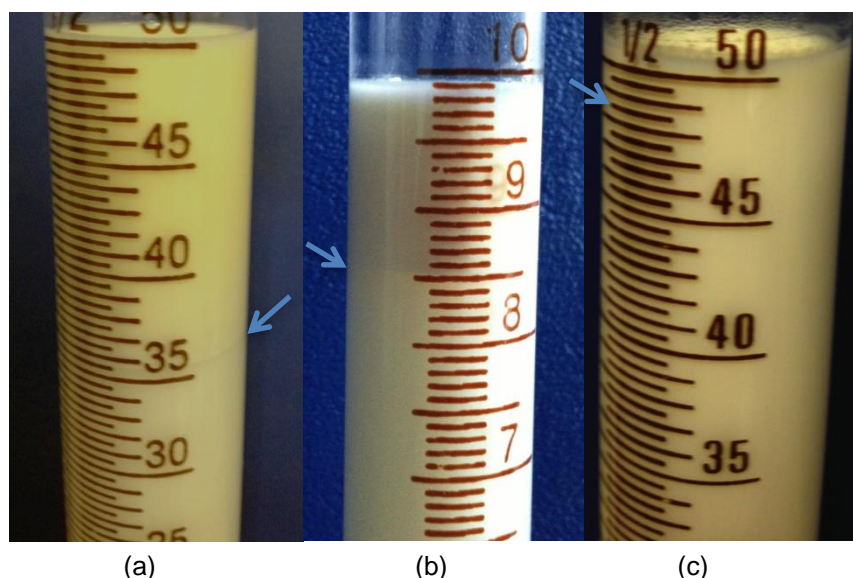


Figure 38. Stability for 7 days of emulsion achieved by turaax emulsification (a), premix ME (b) and direct ME (c)

In spite of all the positive results for premix membrane emulsification, the creaming stability along the time of the emulsion produced by this method did not presented good results, having a great cream formation in just 7 days. As the stability is direct related to the pore size, this could be a reason for such bad results. However, even being much less stable than direct membrane emulsification, both processes using membrane for the production of emulsion showed to be much better than Ultra-Turrax, once they resulted in smaller droplet size with narrower distribution and much larger stability than the coarse emulsion.

4.4 Microencapsulation by spray dryer

From the results of the experimental design, an optimum point was chosen for the encapsulation of linseed oil. The optimum process chosen was CFME 2, with maximum applied transmembrane pressure (4.5 bar), minimum surfactant concentration (1%) and cross flow velocity (3 m s^{-1}). This decision was done based on the observation that the only variable that showed significant difference on the evaluated responses was the transmembrane pressure, having a positive influence on the flux. As none of the other parameters had significant influence, the lower boundaries tested were used in order to decrease process costs in terms of energy and ingredients purchase.

The process was carried out as described before, and after emulsion achievement, CAPSUL (National Starch, São Paulo, Brazil) was added to the emulsion until complete dissolution. The oil:wall material used was 1:2 and it was based on the study made by Barroso (2012) that showed that this wall material at this rate presented very high protection against oxidation for linseed oil. No mechanical stirring was used in order to not modify the droplet size obtained by membrane emulsification.

A very fine powder was obtained and the results for particle size distribution analysis by laser diffraction together with the droplet size distribution for emulsions are shown in Figure 39.

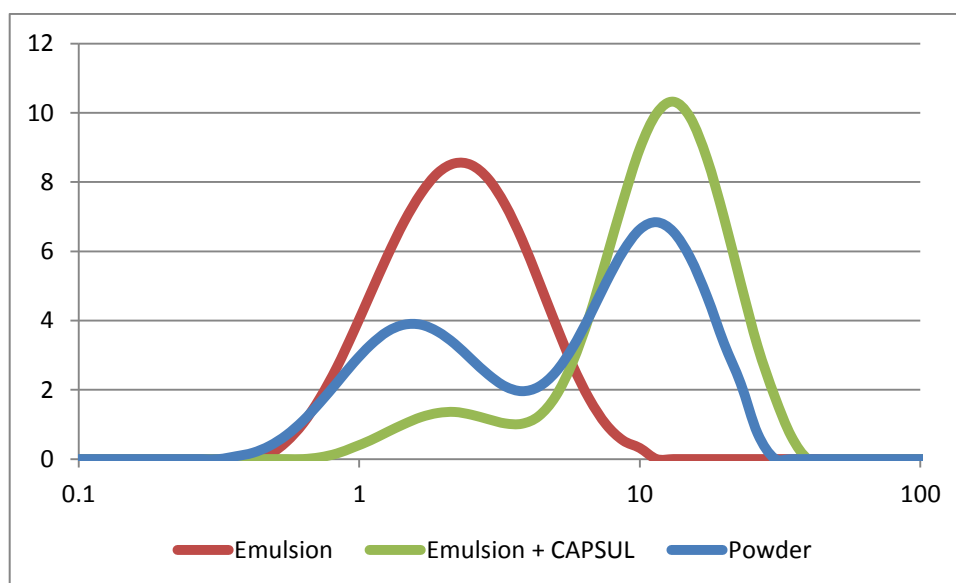


Figure 39. Droplet and particle size distribution for emulsion and capsules achieved by spray dryer

According to Figure 39, the addition of the wall material drastically changed the droplet size and also changed its distribution from a monomodal to bimodal distribution. This indicates that in some cases, more than one oil droplet was entrapped in the wall material. Besides, the capsules formed followed the same behavior as the emulsion with the addition of the wall material (CAPSUL), with bimodal, but much wider distribution. These results were confirmed by the particle size parameters shown in Table 11.

Table 11 .Span, Sauter and De Brouckere mean diameter for emulsion and capsules achieved by spray dryer.

Steps	D[3.2] (μm)	D[4.3] (μm)	Span
Emulsion	2.06	3.28	1.81
Emulsion + CAPSUL	7.81	13.41	1.55
Powder	2.88	8.11	2.43

As shown in Figure 39, the span value confirmed the wider capsules size distribution. Compared to the only paper that got together atomization by spray dryer with membrane emulsification (Ramakrishnan *et al.*, 2012), this result was very good. The authors promoted microencapsulation of fish oil, using two membranes, nylon and mixed ester cellulose, with a mean pore size of 0.8 μm and using Tween 20 as surfactant to promote premix membrane emulsification. In this paper, only Sauter mean diameter was evaluated, and an increase of particle diameter was also observed, but much higher than the obtained in the present work. It was observed an increase on droplet size to the capsule of 6.8 times and 8.2 times with span of 2.20 and 2.37 for the nylon and mixed ester membrane respectively, while in the present work an increase of only 1.4 times was observed in the Sauter diameter.

This small particle size obtained by membrane emulsification followed by spray dryer is very interesting for several applications. Therefore, according to Ramakrishnan *et al.* (2012) emulsions produced by membrane emulsification had less surface oil and yield better encapsulation efficiency, as compared to those produced by rotor stator.

The microscopic analysis by SEM showed that the microcapsules presented spherical shape, however with several depressions on the surface and a wide particle size distribution, confirming the result obtained before. Moreover, these capsules presented a very well structured wall, presenting no cracks (Figure 40). This is very desirable, since the capsule demonstrate to offer a great oil protection, once they have continuous surface and low contact with the air.

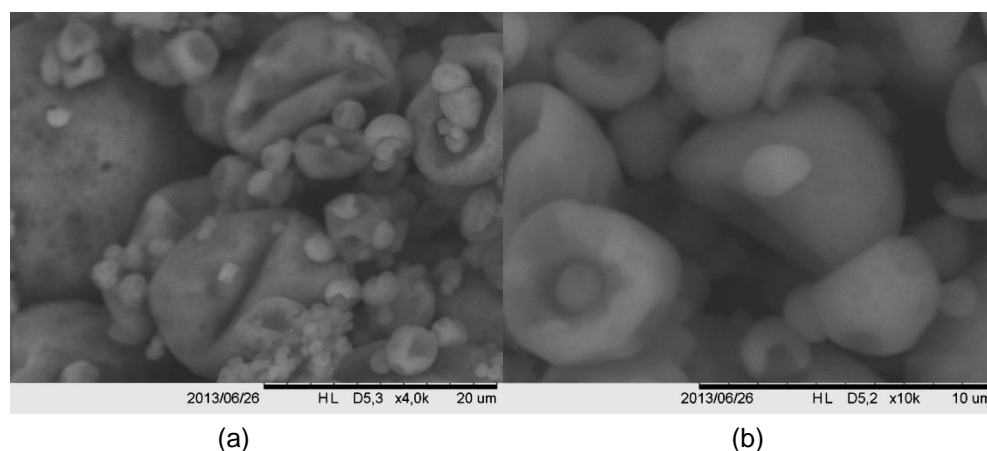


Figure 40. Microphotographs of capsules achieved by cross flow membrane emulsification and spray dryer with magnification of 4000 X(a) and 10000 X.

The depressions were also observed by Barroso (2012), even using different method for emulsion achievement. She proposed that this behavior was due to the wall material used, since other authors reported the same result and the fast particle shrinkage due to the fast drying process. Besides, Teixeira *et al.* (2004) relates that the smaller the particles are, the higher is the surface roughness, since the wall solidification occurs before microcapsules expansion.

5 CONCLUSION AND FUTURE PERSPECTIVES

Linseed oil emulsions were achieved by means of premix and direct membrane emulsification, as well as the capsules achievement.

Polysulphone membrane with pore size of 0.1 μm and cellulose ester membranes with pore size equal or smaller to 0.45 μm showed to not be suitable for emulsion production in the range of tested parameters, since all the processes carried out with these membranes resulted in demulsification.

Pore size showed to be the most important parameter in the range of tested membranes for premix membrane emulsification, since only the mixed ester membrane with particle size of 0.8 μm was able to produce stable emulsions. However, the transmembrane pressure also showed to have great influence on premix membrane emulsification, since low pressures leads to emulsion concentration due to oil droplets retention by the membrane, while high pressures leads to oil droplets passage through the membrane and coalescence. Thus, an optimum transmembrane pressure has to be established for each membrane material and pore size in order to avoid both kinds of demulsification. On the other hand, agitation did not have a great influence on the emulsion production.

The multi-stage premix emulsification showed to decrease the mean droplets size and improve the droplet size distribution. Three cycles showed to be enough to decrease the span value from 1.35 to 0.68.

The cross flow membrane emulsification showed to be suitable for emulsion production with pore size of 0.2 μm , presenting mean droplet sizes in the range of 0.898 to 1.768 μm . Cream formation in 28 days was observed for all the performed tests, thus, studies on different surfactants or even a mixture of them has to be carried out in order to improve this parameter. The experimental design showed that the driving force of the process (transmembrane pressure) was the only parameter that showed significant difference on one of the evaluated responses, the dispersed phase flux. Droplet size, span and stability did not show significant difference between all the processes in the studied ranges. For that reason, the most suitable process chosen was the one that applied higher transmembrane pressure, in order to assure high throughput, and lower velocity and surfactant concentration, due to economic reasons.

Comparing premix and direct membrane emulsification, premix showed to be more suitable for emulsion production in terms of droplet size correlated to pore size and productivity, however, emulsions produced by this method had much lower creaming stability. Thus, for the achievement of emulsions to be used as a food additive in formulations or even being the emulsion the product itself, direct cross flow emulsification showed to be more suitable, since stability is one of the most important parameters to determine the product shelf life.

It was possible to achieve microcapsules by spray drying, with a rough surface without cracks. However, the particle size distribution was bimodal, with span of 2.432. Membrane emulsification showed to be an important process for the achievement of microcapsules, once according to Jafari *et*

al. (2008) smaller droplets are more easily entrapped within the wall matrix of the microcapsule and the emulsions with the smallest droplet sizes are more stable during the spray-drying process.

Future perspectives for this work:

- To evaluate the use of a broader range of membrane types and pore size in order to understand the influence of membrane material on emulsion production.
- To study for each membrane tested its morphology in order to study the pore interconnectivity and to understand the influence of this on emulsion coalescence.
- To study the interactions between the emulsion components and the membrane to overcome them. Moreover, to promote modifications on membrane surface to improve hydrophilicity in order to avoid surfactant interaction and consequent adsorption, once according to Li *et al.* (2009) in the case of O/W separation, hydrophilic materials are less sensitive to adsorption compared to hydrophobic ones.
- To compare the membrane emulsification methods using the same membrane and module in order to remove the influence of the module design and membrane material and its fabrication method.
- To compare membrane emulsification with other conventional systems of emulsions production (high pressure homogenizer, microfluidizer, ultrasonics);
- To produce solid microcapsules by spray drying using different kinds of wall material; To characterize the microcapsules achieved in terms of particle size and its distribution, encapsulation efficiency, porosity and microstructure.
- To encapsulate lipophilic functional components in the lipid matrix to be used as food additive.

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